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THE EFFECT OF PHYSICAL AGENTS ON HYDATID SCOLEX VIABILITY

BY L. B. FASTIER, *The Hydatid Research Department of the New Zealand Medical Research Council, University of Otago, Dunedin, New Zealand*

(With 6 Figures in the Text)

It is the purpose of this paper to determine the effect of heat and of ultra-violet radiations on the viability of the hydatid scolex. Thermal death-rate experiments were designed to test the scolicicidal effect of heating offal prior to dog feeding. This method of offal treatment is practised by some farmers in New Zealand, and it is desirable to assess the effectiveness of different periods of boiling in destroying the scolex at this stage in the hydatid cycle. Ultra-violet irradiation of the scolices *in vitro* was studied to learn whether it would be effective as a scolicicidal agent to supplement or replace formalin in surgery of the human hydatid cyst.

Ross (1929) showed that scolices contained in intact cysts of infected offal were viable for at least 3 days after exposure of such material to the weather experienced in Southern Australia, while storage at a temperature of 4–8° C. failed to impair vitality in 8 days. On the other hand, freezing was found to be lethal (Dew, 1928). The feeding of cooked material to dogs has been widely practised as a method of offal disposal. Dew (1928) stated that heating to a temperature of 50° C. was sufficient to kill scolices, but makes no mention as to whether this figure was obtained from heating free scolices or those contained within infected organs. The susceptibility of scolices in multilocular cysts deeply embedded within sheep organs was also not stated, and the question arises as to the efficacy of this treatment under such circumstances.

Dévé (1905, 1925) and Dévé & Billard (1922) recorded observations on scolices after X-ray treatment at 20,000 r. Although impairment of viability and regenerative capacity were noted, the author urged the reinvestigation of this subject. To my knowledge no such report has appeared. The observations of Jones & Hollaender (1944) and Stowens (1942) on nematode ova and *Trich. spiralis* larvae respectively have shown that, in the former, viability was reduced, and in the latter case impairment of larval invasiveness resulted. Similar investigations upon the hydatid scolex were, therefore, considered of interest.

EXPERIMENTAL METHODS AND RESULTS

Pulmonary and hepatic hydatid cysts were derived from sheep. Fluid was removed aseptically with a syringe bearing a wide bore hypodermic needle and the adventitia dissected to expose the cavity. By gentle suction scolices were removed from the membranes and transferred to a tall measuring cylinder containing 0·85 % saline. After sedimentation for 10 min. the supernatant fluid was removed, replaced with fresh saline and the container vigorously shaken to aid disintegration of brood capsules and to free the scolices. This process was repeated three times, and the final scolex deposit then transferred to a large Petri dish placed over a black tile. After adding a small volume of saline and imparting a rotary motion to the contents, the bulk of separated scolices could be removed with a fine pipette. The resulting suspension of single scolices was then made to a convenient volume with cyst fluid and a known volume withdrawn for counting on a ruled slide. The original 'stock suspension' was then made up with hydatid fluid to contain 4000 scolices in 2·0 ml. for both irradiation and heating experiments.

Two means of estimating viability of scolices were employed. The first depended on a modification of the substage heater devised by Butler & Christenson (1942). By using 4 cm. resistance wire (30-gauge) coiled 1 cm. below the microscope stage aperture and operating on 5 V. a.c. a slide temperature of 60° C. was attained in 10 sec. Scolices were observed on the slide under a magnification of approximately 200 diameters. The criterion of viability was movement of the scolex and, when muscular contraction was marked, the evagination of the rostellum after a single period of heating for 6–8 sec.

The second means depended on the observation that an aqueous solution of eosin (0·006 %), or a 1:2000 solution of neutral red in 0·4 % saline, gave a ready method of distinguishing viable from non-viable scolices. When mixed with an equal volume of scolex suspension and incubated at 37° C. for

5 min. these stains were imparted selectively to non-viable scolices. On closer microscopic examination it was seen that these stainable scolices showed no ciliary movement within their solenocytes, and it was concluded that the property of dye absorption was related to failure of the scolex excretory system. When scolices showing dye absorption and no flame cell activity were transferred to sterile hydatid fluid maintained at 37° C., no recurrence of flame cell movement could be observed, and within one day granular degeneration of the scolex was apparent.

Morphological changes indicative of death, or approaching death, took the form of loss in definition of the refractile elements of the scolex, as well as the extrusion of the 'spherules bulleuses' of Coutelen (1927).

Control cultures of scolices showing well-defined excretory activity and no evidence of dye absorption remained motile for 5 days before evidence of vesiculation occurred. Since stainable scolices were devoid of movement after heating on the stage heater and showed cytoplasmic degeneration, it was concluded that a combination of staining and heating offered a reliable test for viability.

Thermal death-rates were measured in quintuplicate. Five 8 ml. tubes were placed in a thermostatically controlled water-bath, and 2 ml. of standardized scolex suspension were added to each. After 5 min. for temperature equilibrium, the tubes were well shaken, and samples of approximately 0·05 ml. of suspension withdrawn from each tube. These were spread on a 4 × 2 in. glass plate appropriately ruled into five sections. Viable counts on the 50–100 scolices in each sample were made, and the mean percentage viability calculated. Further samples were removed every 5 min. for counting.

The source of ultra-violet irradiation was a Hanovia 'Air Sanitizer' mercury lamp giving an emission of mainly 2537 A. The minimal erythemic dose at 30 inches was obtained from a 5 min. exposure. For irradiation experiments the prepared suspension of 4000 scolices in 2 ml. hydatid fluid was pipetted into shallow sterile dishes where it formed a fluid depth of 2 mm. Exposures were made at distances of 3 and 21 cm. from the arc and for exposure times of 1, 2, 4, 8 and 16 min. Dishes were prepared in duplicate for each degree of irradiation and for unirradiated controls and exposed, covered and then left at 37° C. in the dark. At 30 min. intervals, thereafter, approximately 0·05 ml. of the well-mixed suspension was withdrawn from each dish and counted as above. The average count for the duplicate experiments was expressed as mean percentage viability.

(1) *Effect of heat*

Thermal death-rates were determined *in vitro*, employing hydatid fluid as a scolex suspending medium. Results of a representative experiment are presented in Table 1 and Fig. 1. In most cases the percentage viability of scolices fresh from the cyst varies from 85 to 95 %.

Table 1. *Thermal death-rates of hydatid scolices exposed at four constant temperatures. (Each figure represents an average of five determinations)*

Heating time (min.)	Percentage viability at			
	37° C.	45° C.	50° C.	55° C.
0	85	86	85	84
5	83	86	85	83
10	84	85	81	65
15	83	85	75	35
20	80	84	63	12
25	82	82	55	4
30	83	81	44	0
35	84	78	30	0
40	83	79	28	0
45	84	76	23	0
50	82	71	14	0
55	83	65	5	0
60	84	65	3	0
65	84	63	1	0
70	84	61	0	0
75	83	60	0	0
80	84	56	0	0
85	83	50	0	0
90	82	42	0	0
95	83	35	0	0
100	81	27	0	0
105	82	22	0	0
110	83	22	0	0
115	82	20	0	0
120	82	10	0	0
125	80	3	0	0
130	80	0	0	0

The following brief experiments were designed to measure the penetration of heat through liver tissue. Liver sections of the size indicated in Fig. 2 were prepared, thermometers inserted to the designated depth and the specimen immersed in a water-bath. The temperature of the surrounding water was then progressively elevated and the readings of all three thermometers compared with the bath temperature (Fig. 2).

It will be seen that the early intra-hepatic temperature is approximately 20° C. below that of the surrounding bath and that increase in intra-hepatic temperature conforms to the general increase in bath temperature. After the water boils the time required for the internal temperature to reach 80–90° C. is approximately 20 min. Examination of scolices in cystic livers shows that these conditions are scolicicidal. However, as an additional precaution against the presence of diffuse multilocular cysts, an additional 10–15 min. immersion in boiling water is recommended.

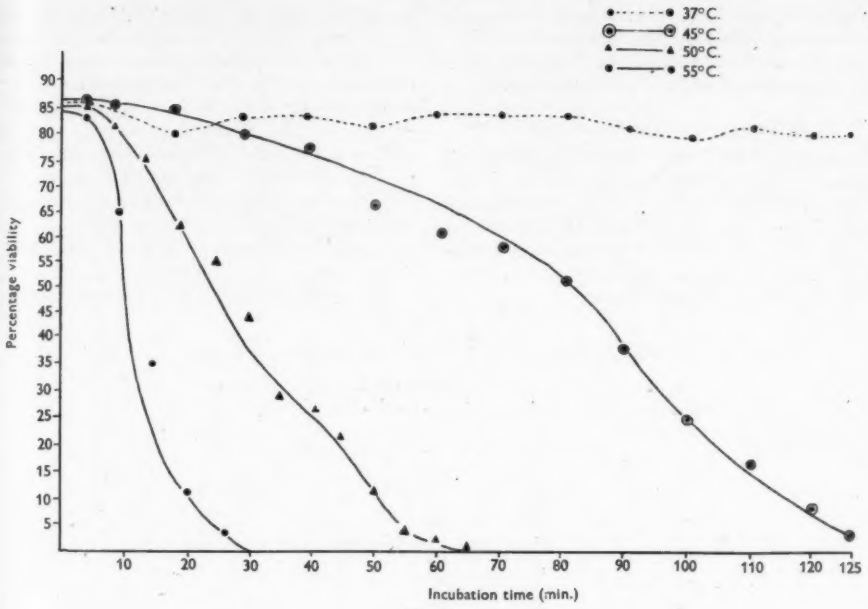


Fig. 1

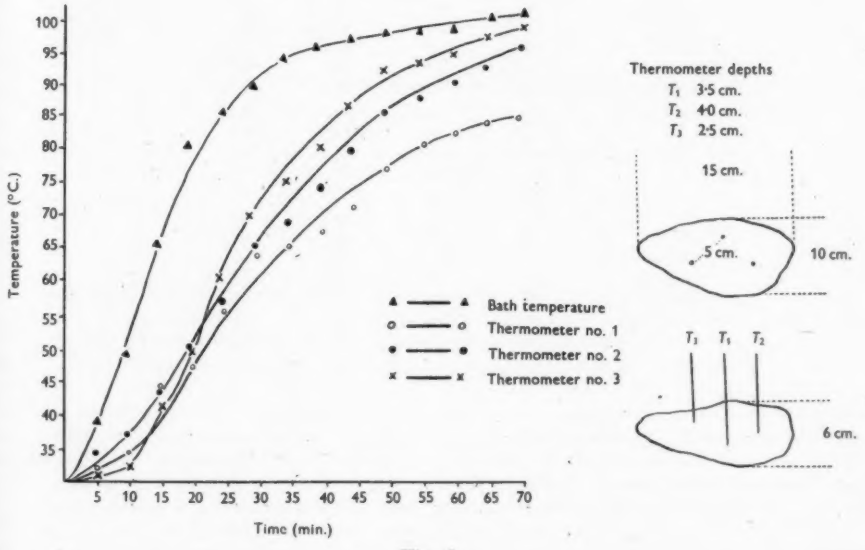


Fig. 2

When similar liver sections are immersed in water at 100° C. the time taken for an internal temperature of 55° C. to be reached is approximately 12 min., and it is not until 30 min. after immersion that an intrahepatic temperature of 85–90° C. is attained (Fig. 3). Observations on scolices in deep-seated cysts indicate that they are not completely destroyed by placing livers in boiling water for 30 min., but that a further 10 min. ensures a margin of safety.

Technical difficulties prevented measurement of

temperature in cyst fluids during heat treatment, but the extent of scolicidal action was estimated by aspiration of cysts and examination of the contained scolices, the results of which are recorded above.

(2) *Effect of ultra-violet irradiation*

Tables 2 and 3 show the change in percentage viability of scolices when maintained at 37° C. following irradiation with 2537 A. at exposure distances of 21 and 3 cm. One duplicate unirradiated control was employed for both experimental series.

Figs. 4, 5 and 6 graphically represent these results and show several interesting features.

bactericidal throughout the suspension; in the latter it presumably prevented air-borne contamination. The nature of the scolicicidal action of the contaminating bacteria is being investigated.

The second feature is that, apart from the above effect, even the intensest radiations used (Fig. 6) were not immediately lethal, and only about one-third of the scolices died from latent effects during the following 13 hr., yet the dosage corresponded to 1000–2000 minimal erythemic doses. At the same time, addition of eosin to the suspending medium did not enhance scolicicidal action.

The third feature is that a small proportion of

Table 2. *Irradiation of scolices at a distance of* 21 cm.

Incubation period after irradiation (min.)	Exposure time at 2537 A. (min.) 1	2	4	8	16
	Percentage viability				
0	92	95	97	96	93
30	90	94	96	93	86
60	89	90	90	90	86
90	84	92	81	86	86
120	87	94	86	84	84
150	86	91	89	82	75
180	84	93	90	81	77
210	85	92	87	81	83
240	83	89	88	82	85
270	89	87	86	84	84
300	89	88	85	83	87
330	88	93	84	88	87
360	88	84	86	88	85
390	88	83	87	88	84
420	85	82	85	88	83
450	87	83	84	89	80
480	85	85	87	85	79
510	87	90	88	86	76
540	88	91	87	85	76
570	89	90	88	86	77
600	82	84	82	87	78
630	75	84	81	84	75
660	69	82	82	86	75
690	67	79	80	82	74
720	69	68	83	76	72
750	68	64	81	74	72
780	60	62	73	73	72

The first is that the control suspensions and suspensions irradiated at 21 cm. for 4 min. or less (Fig. 5) all showed an abrupt loss of viability after subsequent incubation for some 10 hr. Suspensions that were more intensely irradiated (Fig. 4) did not show this effect. Closer microscopic examination showed that bacteria appeared in the controls after 450 min., but were lacking in the more intensely irradiated suspensions of Figs. 4 and 6. Contamination of the suspensions had apparently occurred, either in spite of aseptic precautions taken or else during exposure of the dishes at irradiation. In the former case, the intense irradiation was apparently

irradiated scolices died within 30 min. after the more intense exposures (Figs. 4 and 6). One explanation might be that hydatid fluid is not transparent to these radiations, so that scolices near the surface were killed while the bulk survived. This supposition was tested in two ways: (*a*) The standard sample containing 4000 scolices in 2 ml. hydatid fluid was allowed to sediment completely in its dish. Then the supernatant fluid was removed and the sedimented scolices drained on filter-paper and irradiated. These moist scolices were as resistant to irradiation as were scolices in hydatid fluid. (*b*) The standard sample was mixed with a counted suspension of *Staphylococcus*

Table 3. *Irradiation of scolices at a distance of 3 cm.*

Incubation period after irradiation (min.)	Exposure time at 2537 A. (min.)					
	1	2	4	8	16	Control
	Percentage viability					
0	92	91	92	93	92	92
30	88	84	84	90	86	92
60	86	85	85	87	82	90
90	85	81	88	90	82	89
120	84	82	85	90	82	92
150	86	80	81	83	78	89
180	87	78	83	82	72	88
210	87	81	82	80	72	91
240	84	81	81	80	76	90
270	85	82	81	80	76	89
300	86	78	78	81	78	90
330	84	81	76	82	80	90
360	86	84	82	78	81	91
390	84	86	79	76	82	91
420	88	85	83	77	83	91
450	86	84	84	74	79	90
480	85	82	83	76	81	90
510	81	80	84	76	83	89
540	80	83	80	74	82	85
570	80	84	80	72	78	78
600	84	82	80	68	74	71
630	86	79	77	61	70	70
660	82	80	78	62	66	66
690	84	78	78	62	67	60
720	82	77	74	61	68	61
750	80	79	76	60	69	60
780	78	76	74	60	68	58

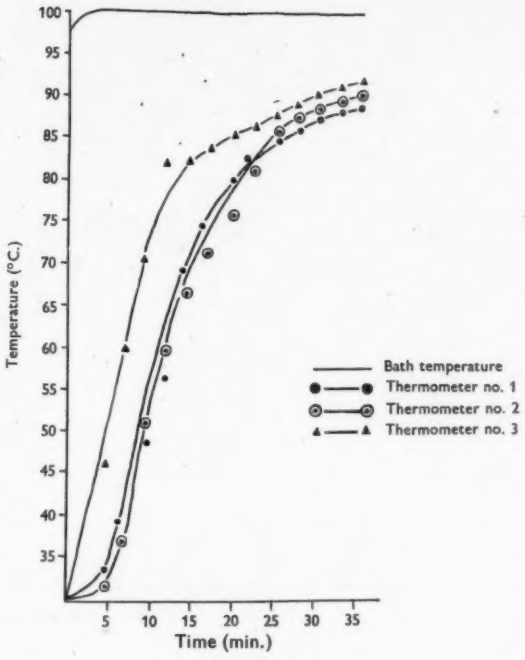


Fig. 3

Table 4. *Irradiation of* Staphylococcus aureus *and hydatid scolices at* 2537 A.

Time of incubation after irradiation (min.)	Unirradiated control		Irradiated specimen	
	Percentage scolex viability	*Staph.* counts (in millions per ml.)	Percentage scolex viability	*Staph.* counts (in millions per ml.)
0	91	6000	90	6000
10	90	6000	89	1900
40	91	7000	89	1800

aureus in hydatid fluid and irradiated for 8 min. at a distance of 3 cm. (equivalent to 1000 minimum erythemal doses). The irradiated suspension and an unirradiated control were then incubated at 37° C. At intervals of 10 and 40 min. respectively, samples were withdrawn for making viable counts on the scolices and also on the bacteria by dilution with medium and plating on agar. From Table 4 it is clear that, when allowance is made for the shadowing of bacteria by scolices, this intense irradiation was markedly bactericidal but lacked any appreciable scolicicidal action. Finally, considerations of shadowing suggest that the accidental contamination noted above was airborne.

DISCUSSION

Studies on ultra-violet irradiation at 2537 A. are interesting in that only slight impairment of scolex vitality results. It is well known that this wavelength lies at approximately the maximal destructive emission for bacteria and, according to Giese (1939) and others, coincides with the point of maximal ultra-violet absorption for certain nucleoproteins. Although a slight, almost immediate, reduction in the number of viable scolices occurs after irradiation there is apparently little latent effect of irradiation as shown by long incubation after exposure. However, after long exposures at 3 cm. distance a definite lethal effect was apparent. The low tissue penetration of rays of 2537 A., the long exposure times involved and the incomplete scolicicidal action would preclude any application in surgery of the human cyst.

Ultra-violet irradiation of hydatid fluid for use as a medium in the tissue culture of scolices might, however, be a ready method for the initial

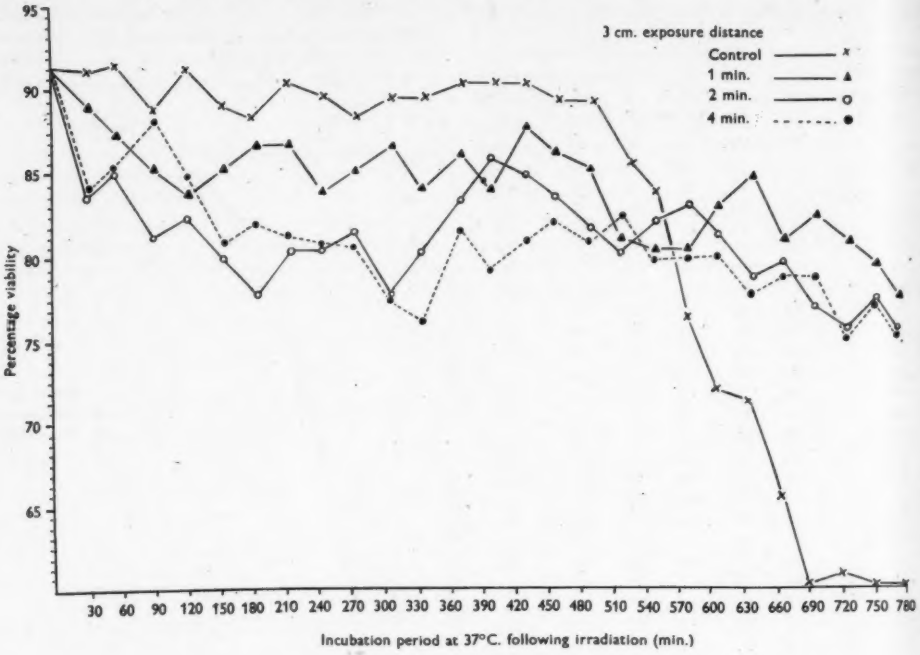
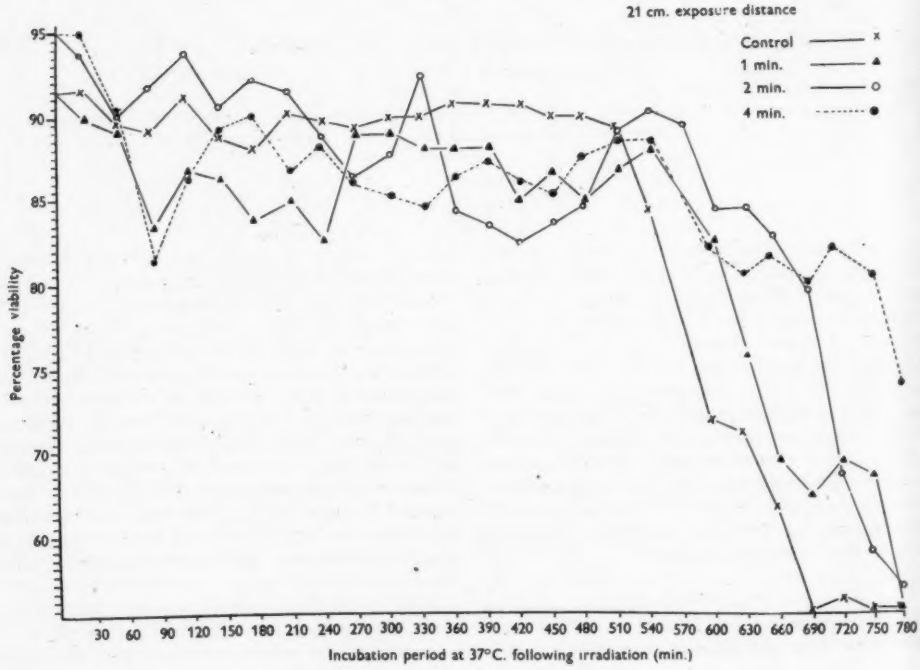

3 cm. exposure distance
Control
1 min.
2 min.
4 min.
Percentage viability
95
90
85
80
75
70
65
30 60 90 120 150 180 210 240 270 300 330 360 390 420 450 480 510 540 570 600 630 660 690 720 750 780
Incubation period at 37°C. following irradiation (min.)

Fig. 4


21 cm. exposure distance
Control
1 min.
2 min.
4 min.
Percentage viability
95
90
85
80
75
70
60
30 60 90 120 150 180 210 240 270 300 330 360 390 420 450 480 510 540 570 600 630 660 690 720 750 780
Incubation period at 37°C. following irradiation (min.)

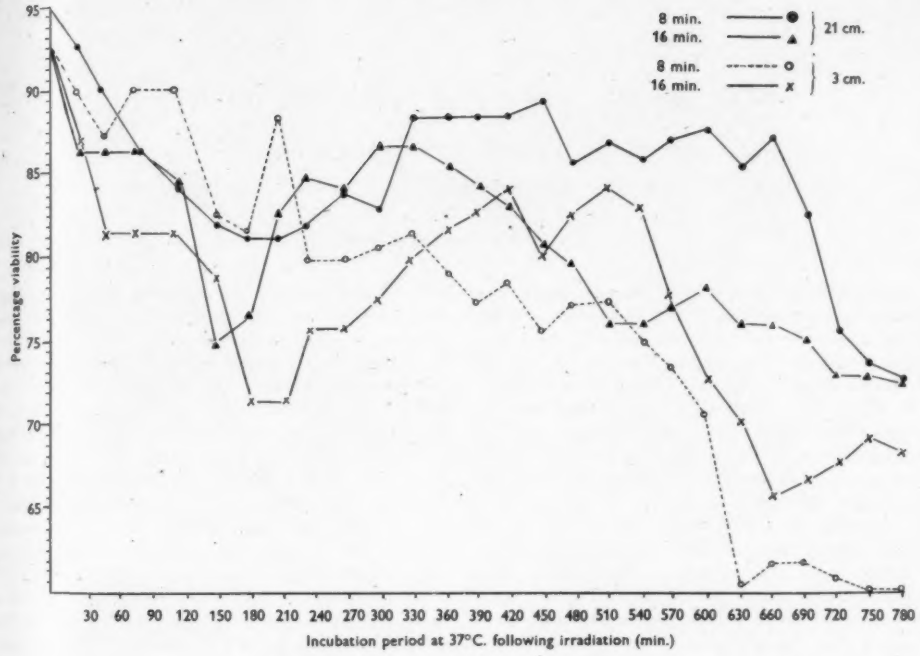


Fig. 6

destruction of bacteria introduced by the process of aspiration, and could be carried out in the presence of scolices without any deleterious effect on their viability. The subject of hydatid antigen inactivation by such radiations has not been investigated, but if no loss in antigenicity occurred this method would offer a ready means of preliminary sterilization of satisfactory fluid antigens for the routine complement fixation test.

SUMMARY

By employing a combination of sub-stage heating and supra-vital staining with eosin or neutral red it was found that viable and non-viable hydatid scolices could be distinguished microscopically. Using this technique a study of the effect of heat *in vitro* on hydatid scolices has shown that death resulted from exposures at 50° C. for 70 min. and at 55° C. for 30 min.

Experiments on the effect of boiling in destroying the contents of fertile cysts embedded in sheep liver showed that when offal was added directly to boiling water destruction of scolices was complete after 40 min. boiling. When the infected liver was placed in cold water brought to the boil, scolex death was assured only after 30 min. subsequent boiling.

Ultra-violet radiations of 2537 A. did not kill scolices under the conditions adopted. Although this wave-length would apparently be ineffective as a scolicicidal agent in surgery of the human hydatid cyst, irradiation of hydatid fluid containing scolices might offer a simple method for initial sterilization of scolex culture media.

The author wishes to acknowledge many helpful suggestions and loan of the Hanovia lamp by Dr G. M. Richardson of the Virus and Immunology Research Department, Otago Medical School.

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STUDIES ON SOME PARASITES OF FISHES

By JAROSLAV WEISER

From the Department of Parasitology, The Charles University, Prague

(With 2 Figures in the Text)

The material described in the present paper was collected by Dr Rašín in 1935. It consisted of an infected ovary of *Leuciscus cephalus* L. from the Svitava river near Brno, and an ovary of *Esox lucius* from Říčka in the vicinity of Vranovice near Brno, C.S.R. The ovaries were fixed partly with formol-Zenker solution, and partly with Gilson's fixative. The infected roes could be distinguished easily from the healthy ones by their white colour. Histological examination showed that they were infected with Microsporidia. As the material had been fixed, no polar filaments could be observed. Sections, 4 μ in thickness, were stained with Heidenhain's iron haematoxylin, which once more proved to be a stain which gave the greatest contrast in preparations of Microsporidia. In addition, sections were stained with Mallory and Mann's stains, and were also subjected to Feulgen's nucleal reaction.

In sections the ovary appeared to be normal. The various follicles were not deformed, and there was no increase in connective tissue. The number of infected follicles found in one section of *Leuciscus* was about 10% of the whole, whereas in *Esox* it was 15%. In the sections follicles were found with young plasmodia creeping in the yolk vacuoles; follicles in which the central part was infected; follicles with an intact membrane and filled with pansporoblasts; and, finally, follicles resembling the atretic follicles in a healthy ovary in which the membrane in some places was dissolved and the inner part infiltrated with connective tissue cells. Such old follicles were found in the tissues as large areas filled with the spores of the Microsporidia. It is apparently from these that the infection by blood or lymph takes place during the resorption of the atretic follicle. As the material was fixed it was not possible to decide whether the spores enter the blood through the wall of the intestine from infected food or mud, or whether the infection is hereditary, spores remaining in the young fish until maturation of the ovary.

The developmental stages of the microsporidian include stages in schizogony and sporogony. The youngest forms are oval schizonts 2–3 μ in size with one or two nuclei (Fig. 1*a*). These can be seen at the beginning of the infection in the vacuoles of the yolk of the egg. The nucleus is compact, and of an irregularly oval shape. Division of the nuclei was seen (Fig. 1*b*, *c*), and daughter nuclei lying opposite to each other were frequently observed, as described by Debaisieux and Kudo. The plasmodia enter into the granules of the yolk where they remain during the whole of their further development. The yolk is used up by the parasite as the latter increases in size forming a hollow sphere which replaces the yolk (Fig. 1*g*). The nuclei are irregular, difficult to observe, and, for the most part, devoid of vacuoles around the compact chromatin granules. In the next stages, which apparently belong to the sporogonic cycle, a clear space is formed around the chromatin granules, representing the inner space of the nucleus (Fig. 1*e*, *g*). The succeeding stages of development stain less well. The large plasmodia divide slowly. Thus the plasmodium shown in Fig. 1*g* has the form of a tripod, the three lobes of which are shown in cross-section, each lobe measuring 2 μ from the common root and 4–5 μ from the free top end. Further division of the plasmodia occurs, and chains of sporonts are formed which may divide further (Fig. 1*i*, *j*). This second division gives rise to the spore. Spherical sporoblasts develop and measure 2–3 to 3–4 μ in size. They have a clearly outlined nucleus which lies free, surrounded by the pansporoblast membrane. During maturation the sporoblasts become elongated (Fig. 1*m*) and no longer stain. A tough envelope is formed round the spores.

The structure of the spore is clearly visible after fixation. There are two vacuoles, one at each end of the spore, and in the middle of the spore is a ring-shaped cytoplasmic body. In the bigger vacoule there is a round granule which stains with Heidenhain's stain instead of the usual small vacuole seen in fresh preparations. In Microsporidia Feulgen's reaction is usually weak, and the nucleus can be seen only with difficulty. In our material, also, the chromatinic substance stained very weakly, and showed a pinkish colour, devoid of sharp outline, in the middle of the spore. In the vacuole at the narrower end of the spore, where the polar filament should be located, a structure was stained with Heidenhain's stain after sublimate fixation, which, taking into account the deformation due to fixation,

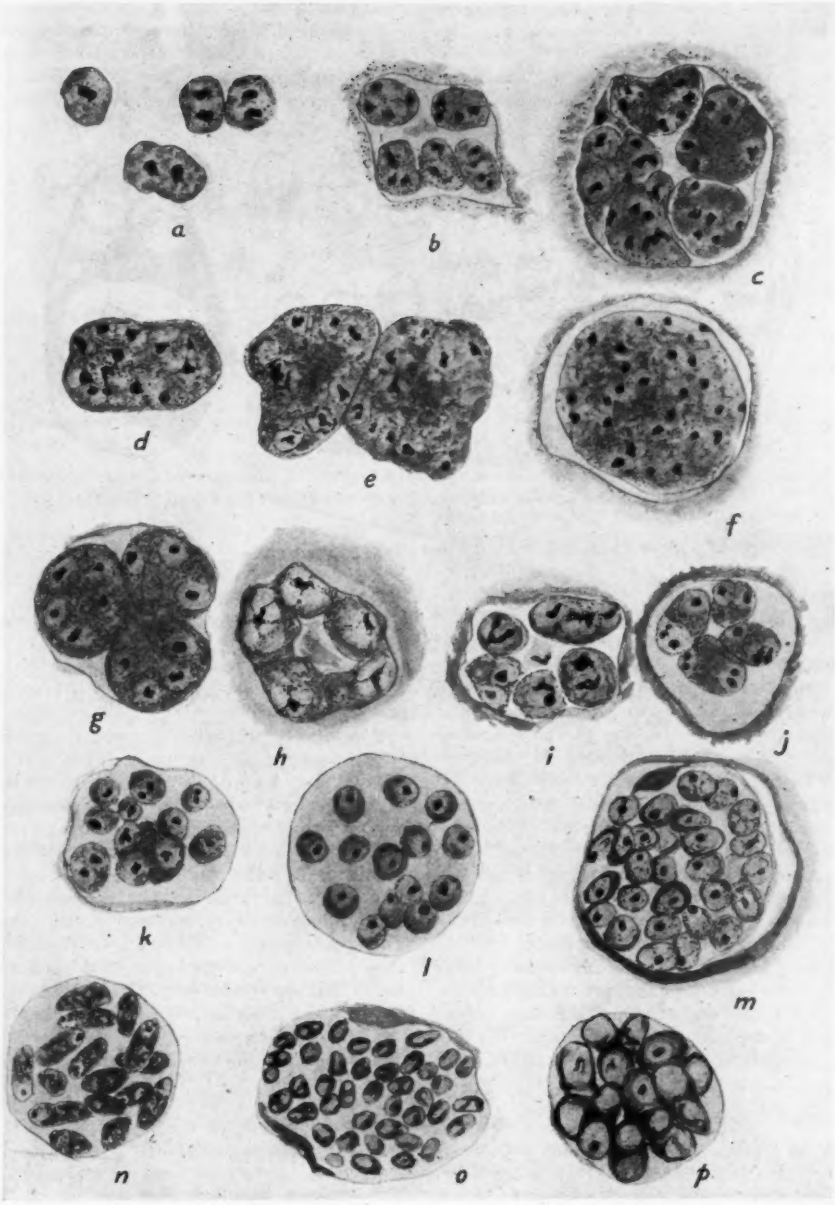

Fig. 1. *a–d*, young plasmodia; *e–g*, plasmodia of the yolk granules; *h–k*, division of the plasmodia into individual sporoblasts; *l–n*, maturation of the sporoblasts; *o*, microsporoblasts; *p*, normal young spores. Magnification × 1000.

is similar to the coiled polar filament. Sometimes only the spine-like middle part is visible, but sometimes traces of the coils can also be seen (Fig. 2*e*).

Three types of spores were found in the material examined. The largest spores, which measured $8{\cdot}4 \times 4{\cdot}2\,\mu$, were situated in the big pansporoblasts.

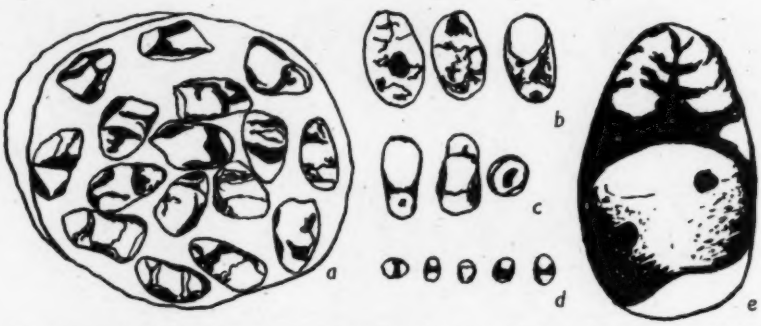

Fig. 2. *a*, mature pansporoblasts; *b*, macrospores; *c*, normal spores; *d*, microspores; *e*, more highly magnified spore with signs of a polar filament. Magnification *a–d*, × 1000; *e*, × 2500.

Medium-sized spores, measuring $5{\cdot}5–6{\cdot}5 \times 3{\cdot}5\,\mu$, were found most frequently. Small spores, $3 \times 1{\cdot}5\,\mu$ in size, were also found filling the central pansporoblasts. The measurements apply to the parasites of *Leuciscus cephalus*. In the pike the spores measured 7×3 to $3{\cdot}5\,\mu$, $5 \times 3\,\mu$ and $3 \times 1{\cdot}5\,\mu$. In this connexion it is interesting to point out that in his description of *Plistophora ehrenbaumi*, the well-known parasite of fishes, Reichenow gave the size of the spores as $3 \times 1{\cdot}5$ to $7{\cdot}5 \times 3{\cdot}5\,\mu$. This parasite is therefore similar in the size of the spores to that described above. However, the Microsporidia show definite specific characters and are also specific for the kind of tissue which they infect. *P. ehrenbaumi*, which produces large tumours in the muscle of the fish *Anarrhichas lupus*, differs in its mode of life from the *Plistophora* described above, which is a parasite of the yolk of the egg of *Esox* and *Leuciscus*.

The parasites of *Esox lucius* and *Leuciscus cephalus* are similar in their development, producing the same three kinds of spores and attacking the same organs of the host under similar circumstances. We are, therefore, entitled to consider them identical. The developmental cycle corresponds to that of the genus *Plistophora*.

Forty-one different Microsporidia are described as parasites of fishes, most of them being found in sea fishes. Only five are known in the ovaries. Thus *Glugia hertwigi* (Weissenberg, 1911) lives in the subcutaneous tissue, cornea, intestine, liver, and in the reproductive organs of *Osmerus eperlanus* and *O. mordax*. The spores are $4{\cdot}6–5{\cdot}4 \times 2{\cdot}3\,\mu$ in size. *Thelohania ovicola* (Auerbach, 1910; Kudo, 1924) was found in the roe of *Coregonus exigurus bondella*. Its pointed spores measure $6–8 \times 4–6\,\mu$. The spores of *Plistophora elegans* (Auerbach, 1910) in *Abramis brama* and *Leuciscus rutilus* measure $10 \times 4\,\mu$. Their meronts live mostly in the connective tissue and they are transported by the blood. *Plistophora mirandellae* (Vaney & Conte, 1901) which lives in the ovary of *Alburnus mirandella*, like our parasite has macro- and microspores, the former measuring $12 \times 6\,\mu$ and the latter $7{\cdot}5 \times 4\,\mu$. Finally, *Plistophora sciaenae* (Johnston & Bancroft, 1919), in *Sciaena australis*, from Australia, forms cysts in the connective tissue surrounding the ovary, and later fills the ovary itself. The spores measure $3{\cdot}5 \times 2{\cdot}3\,\mu$. Although some of these descriptions are somewhat incomplete, it seems that our species cannot be identified with any of the species quoted. We propose, therefore, the name *Plistophora oolytica* because of its faculty of destroying the follicle which it attacks.

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L. HOST RELATIONSHIPS OF THE TICK

PART 1. REVIEW OF PREVIOUS WORK IN BRITAIN

By A. MILNE*
Department of Agriculture, King's College, Newcastle upon Tyne

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1. INTRODUCTION

In the present Part 1 of this paper we review and attempt to synthesize all the previously published information on host relationships of the sheep tick, *Ixodes ricinus* L., in Britain. In Part 2 we set forth our own observations made in northern England. *Ixodes ricinus* ranks as a major pest in British hill farming. Accordingly, though not neglecting the wider ecological aspects, we centre attention largely upon a quantitative comparison of the parts played by wild fauna and farm stock in maintaining the tick populations of hill and moorland grazings.

2. MATERIAL

So far only one author (MacLeod, 1934) has specifically broached the problem of host relationships in Britain. In an experiment on tick control Moore (1938*a*, *b*, 1939, 1942) gave a little attention to the infestations of some of the wild fauna species, with a view to exterminating important hosts as a supplement to acaricidal treatment of sheep. The remaining data comprise scattered and generally more or less weak records from the following authors: Williams (1883), Carmichael (1884), Shipley (1911), Nuttall & Warburton (1911), Nuttall (1916), MacLeod (1932, 1938, 1939), Thompson (1934, 1936, 1937), Elton (1937), Hendrick, Moore & Morison (1938), Cameron (1939) and Prof. J. Heslop Harrison, F.R.S. (unpublished data on *Lacerta vivipara*).

In a number of surveys of ectoparasites from bodies and nests of mice, voles and shrews, *Ixodes tenuirostris* Neumann was the only tick present (see Elton, Ford, Baker & Gardner, 1931; Elton, 1934; Davis, 1934; and Hora, 1934), but we cannot take the absence of *I. ricinus* as significant since the authors do not say whether the localities of these surveys are situated in *ricinus*-infested districts or not.

Note. Unless otherwise stated, the common terms 'tick' or 'ticks' always refer to *I. ricinus* in this paper.

3. BRITISH HOSTS OF *IXODES RICINUS* L.

(i) *Tick stages unspecified*

Some authors do not specify the tick stages found infesting the host; others do. From both we derive the following list of British hosts to one or more of the stages of *I. ricinus*.

Note. The authors' *complete* identical nomenclature is given in every case.

Mammals

Man, horse, cow, sheep, goat, pig, dog, cat, wild-cat, rat, 'deer', red deer, roe deer, 'hares', brown hare, mountain or blue hare (*Lepus variabilis*), rabbit, fox, ferret, stoat, weasel, squirrel, hedgehog (*Erinaceus europaeus* L.), 'bats', 'fieldmice'. Minimum total: 23 mammal species.

Birds

Poultry, capercailzie, pheasant, British red grouse (*Lagopus scoticus scoticus* (Latham)), partridge, curlew, green plover, golden plover, woodcock, 'wild-duck', blackheaded gull (hatched on grouse moor), wood-pigeon, rook, hoodie crow, jackdaw, barn owl, kestrel, merlin, sparrowhawk (*Accipiter nisus nisus* (L.)), starling, siskin (*Carduelis spinus spinus* (L.)). Minimum total: 21 bird species.

* Agricultural Research Council Unit of Insect Physiology.

Reptiles

'Lizards', viviparous lizard (*Lacerta vivipara* Jacquin). Minimum total: 1 reptile species.

With one exception (*Lacerta*) the listed hosts are confined to mammals and birds. But quite a number of wild mammal and bird species, common to typical tick-infested country (hill and moorland grazings, Milne, 1944), are absent from the list. And no British author has mentioned a single non-host. Very probably, therefore, the host list is incomplete. Its variety and length, however, do suggest that, given the opportunity, some stage of the tick will parasitize practically any terrestrial bird or mammal.

identifications of material sent to them. Inexperienced or lay collectors may not have looked carefully for all the tick stages on an animal. The museum workers, of course, make no claims concerning non-occurrence of a stage. Where authors do make such a claim, they very rarely (MacLeod is a notable exception) give the full details necessary to substantiate it, namely, species sample size, date and seasonal tick activity curves for the district. In some cases, therefore, absence of a stage may mean no more than (*a*) inadequate sampling, or (*b*) sampling at the wrong time, or both. (*a*) In any species, infestation variation, among individuals at the same time and place, is so large that its range may include zero

Table 1. *Stages of* Ixodes ricinus *found on individual species of vertebrates* (*from published work*)

♂♂, males; ♀♀, females; oo, nymphs; LL, larvae. p, stage recorded present, with qualification in case of * (see text); –, recorded absent, or more usually, not mentioned. Figures in parentheses denote known (published) numbers of specimens examined.

	♂♂	♀♀	oo	LL
Mammals				
Man	p	–	–	p
Cow	p	p	–	–
Sheep	p	p	p	p
Dog	p	p	–	–
Cat	–	p	–	–
Rat (1)	–	p	–	–
Red deer	p	p	p	p
Roe deer	–	p	–	–
Brown hare (5+)	p	p	p	p
Mountain hare	–	p	–	–
Rabbit (5+)	–	p	p	p
Fox (4+)	–	p	p	p
Stoat (2+)	–	–	p	p
Common squirrel	–	–	p	–
Hedgehog (9)	p	p	p	p
*Fieldmice spp.?	–	–	p	p
Birds				
*Poultry	–	–	p	p
*Capercailzie	–	–	p	p
*Pheasant	–	–	p	p
Grouse (49)	–	p	p	p
*Partridge	–	–	p	p
*Curlew	–	–	p	p
*Green plover	–	–	p	p
Golden plover (2)	–	–	–	p
*Woodcock	–	–	p	p
*Wild-duck spp.?	–	–	p	p
*Blackheaded gull	–	–	p	p
*Wood-pigeon	–	–	p	p
Hoodie crow (3)	–	–	p	p
*Rook	–	–	p	p
Jackdaw (4)	–	–	p	p
Barn owl (1)	–	–	p	–
Kestrel (2)	–	–	p	p
Merlin (1)	–	–	–	p
Sparrowhawk (1)	–	–	p	p
Siskin (1)	–	–	–	p
Starling (2)	–	–	p	p
Reptiles				
Viviparous lizard	–	–	p	p

(ii) *Tick stages specified*

In Table 1 more than one author may have recorded for a particular species. This is not indicated, because we are not always clear whether one author is merely quoting another. The table gives the complete available published data on tick stages infesting individual species in Britain.

If a tick stage is recorded present we are on sure ground, except in the case of some of Moore's data marked *. He gives these animals as a group yielding nymphs and larvae only, as opposed to adult ticks, but one or other of the immature stages may have been absent.

On the other hand, where tick stages are absent in the table, there is room for doubt. In the first place, some of the data arises from museum workers' during high tick activity (Milne, 1943, and Part 2 of this paper). Collecting a representative sample of a species is often laborious and difficult, and in the case of most birds illegal. We imagine, therefore, that the specimens examined were generally too few, probably only one for some species. (*b*) Activity times of the different tick stages do not coincide, though they overlap to a greater or lesser extent. Thus, there are times when only adults are active, and times when all stages except larvae are active. This fact has only recently come to light (Milne, 1945*a*, 1947*a*). Further, activity times for the same stage differ in different regions of Britain (Milne, 1945*a*). Points (*a*) and (*b*), together with the incompleteness of the host list, allow opportunities for wrong conclusions. The above considerations are even more important in quantitative work.

(iii) *Difference in the infestations of mammals and birds*

MacLeod (1932) makes the point that 'only immature (tick) stages were found on birds'. After listing a number of birds on which larval and nymph stages only had been found, Moore (1938*a*) says: 'There are recorded [by himself] 5 cases in grouse where one adult female tick has been found. The occurrence of adult female ticks on birds appears to be accidental rather than regular, and we take the view that the life cycle of the tick cannot be completed with bird life only. Generally speaking, infestation of animals [mammals?] is by larval and nymph stages and adult female ticks.' Cameron (1939), without giving any data, states: 'among wild hosts, numerous birds and mammals have been recorded. As a rule, birds are parasitized only by the immature stages, larvae and nymphs, of the tick, whilst all stages, including the adult male and female, occur on mammals.' In Table 1 we have seen the total (and in some respects, unsatisfactory) published data on which this idea could be based.

Note. The apparent relative scarcity of male ticks (Table 1) is no doubt due to the fact that males, being almost always unattached to the host, are less easily seen than the larger, anchored females, except when *in copula*.

4. HOST POTENTIALS OF INDIVIDUAL SPECIES

Having now some idea of the hosts for immature and adult ticks, the next question is: what proportion of the tick population on a typical hill grazing is supported by each of the available host species? To answer this reliably we require the average seasonal total of ticks per individual for each species together with species population densities. Except for our own work in hill sheep (Milne, 1947*b*, and Part 2 of this paper) no precise data of this nature have been published for *Ixodes ricinus* in Britain, nor indeed in any country so far as we can ascertain. Two authors, MacLeod and Moore, give tick counts on wild animals implying comparison, but not actively comparing, with the order of tick counts on sheep. The following is the total information we have managed to put together for these authors.

Moore (1938*a*) gives three rather inadequate counts for forty-nine adult red grouse, *Lagopus scoticus*, on Hill of Fare, north-east Scotland. With a little trouble, these may be roughly compared with counts for sheep (also in the paper) for the same times and place, as follows (average female ticks per individual, with number of individuals per sample in parentheses):

Grouse	Sheep
1 (1)	23 (6)
0 (1)	15 (6)
0 (47)	*c.* 10 (6)

These data suggest that as host to female ticks the average grouse is negligible compared with the average sheep. Lovat (1911) gives the highest normal grouse density in Britain as one nesting pair to 2 acres, while the highest normal sheep density on heather (grouse habitat) is about one sheep to 4 acres, i.e. only four grouse to one sheep. Therefore, as normal adult populations, the host potential of grouse must also be negligible compared with that of sheep.

Moore (1938*a*, *b*) claims that, on heavily infested land in north-east Scotland generally, the Scottish mountain hare, *Lepus timidus*, carries 'large numbers' of female ticks. Indeed, as an adjunct to other tick control measures on certain sheep grazings, he has almost exterminated the hare population. Unfortunately, he has published no tick counts for hares. Gamekeepers say that more than one hare to 5–10 acres is not permitted on a well-managed moor. And on moors frequented by the mountain hare, sheep will certainly not exceed one to 4 acres, indeed one to 10 acres should be nearer the mark. That is, about one hare to one sheep. But neglected moorland sometimes becomes overrun with hares. Thus, for example, on the 6000-acre Hill of Fare 'from April 1937 to the end of January 1938, they killed 1243 hares' while the sheep stock totalled only 500 (Moore, 1938*a*). In the nature of things we would expect the individual sheep to carry far more female ticks than the individual hare. But 'large numbers' on hares in conjunction with the density figures just given would suggest that, as populations, hares may make a not inconsiderable contribution to the support of female ticks on a hill grazing. The matter should be investigated.

On red deer, *Cervus elaphus*, MacLeod (1938) states: 'Tick infestation of deer may run to 500 or in extreme cases nearly 1000 females per deer.' In a letter, dated 26 January 1947, MacLeod further informed us: 'I made no counts on red deer but merely accepted the unpublished figures which Moore at that time gave.' Moore (1938*b*) states: 'Perhaps the greatest problem in [tick] control [in certain areas of north-east Scotland] is [red] deer. These animals may have thousands of ticks attached....' In this case Moore was referring to conditions in the then grossly overstocked deer forest of Glendye. The existing tick-count data for deer are, therefore, due entirely to Moore. Now we showed (Milne, 1947*b*) that the maximum average ticks per sheep in Britain at peak activity is about 150 females, with individual maximum ranging up to about 300. We may conclude, therefore, despite the vagueness of Moore's figures, that the individual red deer has a higher comparable infestation weight than the individual sheep.

Stocking densities, of course, depend on the grazing value of the land. On the question of normal maximum stockings, Mr F. Wallace, Deer Forest

Control Officer of the Department of Agriculture for Scotland, states, in a letter dated December 1945: 'I should say 4 red deer to 100 acres is not excessive on natural deer ground.' On the other hand, Darling (1937) believes that one deer to 30 acres is verging on overstocking in the north-west Highlands of Scotland. The corresponding figure for sheep in deer country would be getting on for 10 acres per sheep. That is, roughly two or three sheep would replace one deer on a grazing. At that rate, the deer might well rival the sheep population in host potential.

MacLeod (1938) contends that the part played by deer on sheep grazings is negligible. He maintains that: (*a*) The red deer's traffic in ticks is largely confined to its lairs. Hence 'the role of resident deer relative to that of grouse, hares and vermin is unimportant' in the maintenance of a deer-forest tick population. (*b*) Sheep and deer seldom share the same grazing, the deer generally being confined to their own 'forests', usually high moorlands and wastes. When deer do invade sheep grazings, it is usually in winter when ticks are more or less inactive. Hence deer must have even less effect on the tick populations of sheep grazings than they have on those of their own forests. 'An obvious argument to support this', he says, 'is that in Northumberland and the Scottish Borders, where there are no deer, the tick-infestation level [of sheep] is actually higher than in the Scottish Highlands, over which the deer roam at will.'

Several criticisms are pertinent. First, in (*a*): As yet, no one has actually compared unfed-tick density on lairs and on general grazing of deer. Hill sheep also have the lairing habit to some extent, lying at night on the same confined areas near the hill tops, all the year round. Yet this does not hinder their building up a high unfed-tick density on their general grazing (Milne, 1946). We cannot therefore agree, in the absence of facts, that the deer's relations with ticks are practically confined to their lairs. Secondly, in (*b*): We know that when deer are overstocked, they do, if fences are inadequate, raid neighbouring sheep runs during the tick activity season, e.g. Glendye, north-east Scotland. Thirdly, in (*b*): The comparatively low sheep-infestation level of the Scottish Highlands could be due not to the innocence of deer but to light sheep stocking. Infestations are lower the lighter the stocking (Milne, 1947*b*). And sheep stocking is much heavier in the Border country, being 1–4 acres per sheep as compared with 4–14 acres in the north. Lastly, in (*b*): Deer forests are tick reservoirs, and a deer fence, if present, is no hindrance to other members of the wild fauna. According to Mr Wallace (in letter cited), the 1939 red deer population of Scotland (very much reduced by 1945–6 owing to war and abnormally severe winters) was about 200,000, i.e. probably equivalent to about half a million sheep with respect to female ticks (see opposite column). The sheep population (all ages) of the Scottish hill lands varies between 2¼ and 4½ millions throughout the year (Cmd. 6494, 1944). We see, therefore, that deer must play quite an important part in maintaining the tick population of Scotland as a whole.

To sum up: The inadequacy of the data is such that we place little confidence in most of the findings in this section. Indeed the section would have been excluded, but for the fact that it suggests, however weakly, the possible importance of Scottish mountain hare and red deer populations as hosts to ticks on the hill and moorland grazings. The suggestion is very tentative and should be looked on more as a spur to adequate investigation of these animals throughout Scotland. In certain areas of north-east Scotland, red deer, roe deer and hares have already been slaughtered because of their alleged importance as alternative hosts (see the works of Moore, listed above). Our wild fauna is far too small to justify uncritical acceptance of a country-wide necessity for this drastic policy. MacLeod (1938) supports this view.

5. EFFECT OF HOST REDUCTION ON TICK POPULATIONS

(i) *The experiment of MacLeod*

In an attempt to elucidate the 'part played by alternative hosts in maintaining the tick population of hill pastures', MacLeod (1934) fenced a 10-acre experimental area of typical Scottish hill sheep pasture with 'game-proof wire-netting of 1½ in. mesh, so that only the smaller forms of mammalian life could obtain ingress'. By grazing this area with sheep dipped in acaricidal material at appropriate intervals, he claims that in 18 months 'the individuals of the original tick population, except those which fed on alternative hosts, were either removed and killed by the repeated serial dippings, or starved to death for want of a blood meal'. 'For the succeeding 18 months, the area was left untouched' [i.e. no sheep or other farm stock grazed on it], 'to allow of the vermin- and bird-supported population attaining an equilibrium.' At the end of the 3 years, ten sheep, freed of ticks, were placed in the experimental area. The average females plus nymphs per sheep was: experimental area, 3·38; surrounding control area, 19·06. MacLeod's main conclusion was: 'It would appear therefore that in the central (i.e. experimental) area a tick population of appreciable size was maintained by ground vermin and birds, although no ticks in this area had engorged on sheep for the previous three years.' He adds, 'The result is the more striking in that the number of possible alternative hosts was very much reduced: hares obtained access (to the experimental area) by

virtue of their leaping powers; apart from them, the only mammals likely to have gained entry, and which are known to harbour ticks, were weasels and stoats. It is not known whether these ticks (*Ixodes ricinus*) feed on mice and voles. The only other possible hosts were the common moor birds.' And hence, justifiably, he claims that 'removal of the chief host (sheep) from a particular area would not effect eradication of the parasite from that area'.

There can be no doubting MacLeod's main conclusion. His data suffice to uphold it. But it is worth while to consider the possibilities of his method in the more precise quantitative field of host relationships.

MacLeod does not give sheep stocking density on the control area. That figure would enable us to estimate the true degree of difference represented by the sample means 3·38 and 19·06 ticks. For instance, if stocking density on the control area were lower than on the experimental then the difference might well be less than appears (cp. Milne, 1945*b*).

A more valuable result would accrue if no member of the wild fauna were barred from the experimental area, i.e. a measure of the relative parts played by sheep and total wild fauna, instead of an unknown fraction of the latter. The main drawback, however, would still remain, namely, environmental changes (cp. Milne, 1944, for effects) in the experimental area due to lack of grazing by sheep. In State afforestation areas of northern England moorland the ground vegetation becomes knee-deep or even waist-deep comparatively soon after the exclusion of sheep! This might be got over partially by periodic mowing, but the latter would not keep the two environments closely similar, since the sheep is a selective grazer preferring the finer grasses and leaving great tufts of untouched rough grasses in between. Moreover, even were the drawback wholly overcome, the method would tell us nothing of the individual host potentials of different species. This is a most important point if anti-tick treatment of stock is to be supplemented by elimination of certain wild species in attempts at control (see Moore, below).

(ii) *The experiment of Moore*

At the beginning of Moore's (1938*a*, 1939, 1942) experiment there were more than 1200 Scottish mountain hares and probably at least thirty roe deer (*Capreolus capreolus*) on the 6000-acre Hill of Fare. The sheep stock was 500. As mentioned before, Moore claims that hares and roe deer were very heavily infested with female ticks. In 6 years he produced an apparent reduction from forty to less than five female ticks per sheep (average seasonal maxima). His method was a combination of serial anti-tick treatment (dipping) of sheep and reduction of alternative hosts. Hares and roe deer were practically exterminated, totals of 3044 hares and more than seventy-five roe deer being killed over 5 years.

Moore appears convinced that sheep, hares and roe deer were the chief hosts for female ticks on this hill. It is a great pity that he did not publish details of tick counts on hares and deer. His measure of tick population reduction is derived from counts of six sheep only, rather a small sample-size for precision (cp. Milne, 1943). However, the consistent gradual lowering of sheep infestations over the 6 years does suggest that his reduction claims are justified. The difficulty for us is that we cannot assess how far dipping and how far wild fauna elimination were responsible. Moore did not dip so frequently as MacLeod, hence a proportion of the ticks on sheep escaped killing. Therefore the tick population remaining after 6 years was composed of some ticks maintained by sheep and others by the wild fauna less the majority of deer and hares. This again tells us little about the precise quantitative parts played by wild fauna and farm stock in maintaining the tick population on a grazing.

6. SUMMARY

On the whole published data on the host relationships of *Ixodes ricinus* L. in Britain are rather inadequate, but the following points emerge:

The tick (stages unspecified) has been found on twenty-three mammal, twenty-one bird and one reptile species. These include both wild and domestic animals. There are no records of non-hosts. The host list is incomplete. Its length and variety suggest, however, that the tick will feed on practically any mammal or bird it may meet.

Tick stages found on fifteen mammal, twenty-one bird and one reptile species have been recorded. For a number of reasons (see text) 'recorded' absence of any tick stage from an animal is not necessarily reliable. This, together with the incompleteness of the host list, detracts a little from the assertion of several authors, namely, that in general larvae and nymphs occur on both mammals and birds, but adult ticks on mammals only.

No precise host-potentials for any wild species relative to sheep (or other important farm stock) have been published in Britain. But, in one area, Scottish mountain hare, red deer and roe deer have been slaughtered on a large scale because of their alleged importance as hosts. Very inadequate data from two authors seem to suggest that as normally stocked populations (*a*) grouse are negligible compared with sheep as hosts to female ticks, and (*b*) mountain hare and red deer may play quite an important part in maintaining female ticks. Suggestion (*b*) at least should not be taken seriously until comprehensively investigated.

One author shows that an 'appreciable' tick population was maintained on a hill grazing by a reduced wild fauna in the absence of sheep.

Another author shows that the tick population of a grazing was considerably reduced by acaricidal treatment of sheep, together with almost complete extermination of a section of the wild fauna. Unfortunately, the two factors cannot be disentangled.

We would like to thank Mr Charles Elton, Director, Bureau of Animal Population, Oxford, and Dr J. MacLeod of the Cooper Technical Bureau, Berkhamsted, for criticism of the MS.; Mr F. Wallace for information on red deer stocking; and Dr V. B. Wigglesworth, F.R.S., Director, Agricultural Research Council Unit of Insect Physiology, Cambridge, within whose organization the work was done.

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L. HOST RELATIONSHIPS OF THE TICK

PART 2. OBSERVATIONS ON HILL AND MOORLAND GRAZINGS IN NORTHERN ENGLAND

By A. MILNE*
Department of Agriculture, King's College, Newcastle upon Tyne

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1. INTRODUCTION

On British hill and moorland grazings the two most important tick hosts among farm stock are sheep and cattle. The numbers of cattle, however, are negligible compared with sheep (Cmd. 6494 and 6498, 1944; Wheldon, 1941). The present work was undertaken primarily to explore the control possibilities in serial anti-tick treatment of farm stock; obviously, the degree of success in such control attempts will depend largely on the relative parts played by farm stock and wild fauna in maintaining tick populations on grazings. Even without a practical bias, however, work on host relationships would, as will appear, inevitably lead to a comparison of wild and domestic hosts.

Part 1 (1948) of this paper shows that the wild fauna does contribute, probably substantially in the case of a few species, to the maintenance of ticks. The precise quantitative relation between contributions from farm stock and wild fauna has not, however, been worked out.

On a grazing we might attempt to assess the parts played by farm stock and the entire wild fauna by MacLeod's (1934) method, modified as suggested in Part 1 of this paper; that is, simply by comparing tick populations on two areas within the grazing, one open to wild fauna only and the other open to farm stock as well as wild fauna. But this has certain practical drawbacks which might even interfere with the end-result (see Part 1). Moreover, the findings for any one grazing would have a somewhat

* Agricultural Research Council Unit of Insect Physiology.

limited general significance, since faunal composition, both as regards species and species density, varies from place to place; nor would results show which wild species are most important as hosts, if reduction of alternative hosts were contemplated. Accordingly, we chose to approach the problem from the species angle.

The work occupied four tick seasons, 1943–6, the bulk being done in 1944.

Note. As in Part 1, unless otherwise stated, the common terms 'tick' or 'ticks' refer to *Ixodes ricinus*.

2. THE GRAZINGS

The main work was done at Lordenshaws, a 1285-acre hill sheep farm on the Simonside range of hills near Rothbury, Northumberland. This farm rises from about 500 ft. above sea-level in the east to 1182 ft., the Beacon, in the west. It is roughly triangular in shape and lies on the Fell Sandstone (see Milne, 1944). The grazing consists almost entirely (*c.* 96 %) of rough heather, including fairly large areas of encroaching bracken, small areas of cottongrass, and some outcropping rock. Variations from this are minor, less than 50 acres in all (4 %): two poor quality hay meadows, a bracken-infested rough-grass field and a fairly mature spruce-fir plantation, all enclosed and lying close round the farm buildings which are towards the centre of the farm; three areas of unenclosed scrub woodland, chiefly birch-hazel with underlying grass or bracken, two lying to the south of the buildings and one on the extreme northern edge of the farm; lastly, an enclosed rough-grass field on the extreme southern edge. Several small streams drain the moorland. The stock is Blackfaced sheep, having access to all enclosed areas except the spruce-fir plantation and, in summer only, the two hay fields. For a considerable distance beyond the boundaries of the farm the land is similar, i.e. rough heather with some bracken, except to the south-east where, apart from two small conifer plantations, the land is enclosed rough grass.

Additional observations on a smaller scale were made on the following places in Northumberland, Cumberland and the Scottish Border:

Hethpool, on the College Water near Cheviot, a 1124-acre hill farm carrying Cheviot sheep with, in summer only, some cattle. No heather grows here, the pasture being dominated by rough bent grass, principally *Nardus stricta*, with large areas of encroaching bracken. Within the grazings are five small conifer plantations, 'shelter belts', fenced against sheep, and one area of scrub oak open to sheep. Some deciduous trees and a good deal of gorse line the river and streams passing through the farm.

Byrness Forestry Area, on the Rede Water above Otterburn, several thousand acres of young conifer plantations and hill farms, the pastures being both rough grass and heather with some bracken, chiefly grazed by Blackfaced sheep. The usual small areas of scrub-woodland and bushes are dotted here and there on the pastures, while bushes with occasional deciduous trees line the river and streams.

High Thorneyburn, on the river North Tyne, a large hill farm, chiefly rough grass with some bracken and a small area of heather, grazed by Blackfaced sheep and some cattle. Perhaps 1–2 % of this farm consists of woodland or scrub, while again trees and bushes line the North Tyne.

Parkhead and Mainsrigg, Whitfield, on the high land south of the South Tyne, two neighbouring hill sheep farms, chiefly heather (60–70 %) with bracken, and some rough grassland (30–40 %). They are grazed by Blackfaced sheep and some cattle. Both farms have about 100 acres of conifer woods lying against their boundaries, i.e. lining about 1½ miles of the 8-mile boundary in each case. Both farms have also one or two small areas of conifer plantation within their grazings: Parkhead, 14 acres and Mainsrigg, 7 acres; and both have the usual small scattered areas of scrub-woodland and tree-bush-lined stream.

Data on wild fauna population densities were also collected from Crag, a heavily tick-infested fell farm in Eskdale at the southern end of the Lake District. This farm, grazed by Herdwick and Swaledale sheep, has some enclosed rough grassland, and rights on the common fell where stocking is about one sheep to four or five acres. The fell consists mainly of rough grass-bracken-bogmyrtle, but poorish heather covers a not inconsiderable area. On the grazings the usual small areas of scrub-woodland, bushes, etc. occur.

A colleague, J. Allan Campbell, kindly supplied data on reptiles from Traquair Knowe, a heather-roughgrass-bracken hill farm near Traquair-on-Tweed. This farm carries a Blackfaced stock of sheep.

The above places are a fairly representative sample of hill sheep farms in the north of England and the Border Country.

The common impression of hill and moorland grazing country is of a treeless 'waste' of rough grass, heather and bracken. Indeed, about 97 % of a typical hill farm is grass or heather, with bracken. But we would draw attention (as above) to the invariable 2–3 % made up of small plantations, small scrub areas, smaller aggregates of trees or bushes, or individual trees and bushes, scattered within the typical grazing. These are often sufficient to provide niches for some wild birds and mammals not strictly associated with open hill and moorland grazings but, nevertheless, coming into the tick picture. We shall show later (§§ 9 and 10) that, for example, pheasants and some small wood-loving birds, roe deer, squirrel, etc., contribute to the maintenance of tick populations on hill-sheep grazings.

Typical seasonal activity curves for all stages of

ticks are given in Milne (1945*a*, 1947*a*) for Hethpool, Lordenshaws and Crag; activity at Byrness, High Thorneyburn, Whitfield and Traquair is similar to that at Lordenshaws and Hethpool, except that in some cases there is rather more emphasis on the autumn recrudescence.

3. METHODS IN GENERAL

In tick control by treatment of farm stock, the method is to concentrate on killing female ticks. There are several reasons for this (Milne, 1945*c*), but the two most important are (*a*) fewer treatments are required since the female stage remains attached to the host about twice as long as the other stages, (*b*) attacking the female hinders multiplication. Our main intention, therefore, was to ascertain how far females were supported by the wild fauna in comparison to farm stock, but, incidentally, we counted nymphs and larvae. The standard for comparison was the sheep, chief farm animal on hill and moorland grazings (see § 1). Put briefly, we compared the average individual of each wild-host species with the average sheep; whence, with a knowledge of species population densities, we compared the contributions of the different host populations to the maintenance of ticks on a grazing.

The first step was to ascertain the hosts. The next step was quantitative. With tick activity fluctuating during the season (Milne, 1945*a*), and with ground density of ticks varying from grazing to grazing (Milne, 1944, 1946), the tick count of one host can be compared with that of another only if both hosts are taken at the same time from the same place. Further, we can only sample the fauna and this implies need for repetition. The best way of comparing would be to make tick counts on adequate live samples of each host species in the fauna at requisite intervals throughout the tick-activity seasons. Returning the animals to the ground after each count would ensure that the natural 'balance' between the tick population and its different hosts was maintained, thus avoiding possible distortion in subsequent counts. This method applies quite easily to sheep, but was not feasible with the wild fauna because, for obvious reasons, most of the wild animals had to be killed before examination.

Removal (killing) of some host individuals should tend, within limits, immediately to raise the absolute infestation level of all host individuals remaining, irrespective of species, since the available ticks are now shared among fewer individuals (cp. the result of suddenly increasing sheep stock tenfold, Milne, 1945*b*). But the relative infestation levels (average individual) among species would remain unchanged if the same proportion of each and every species population were withdrawn at a sampling. Unfortunately, such a nicety was again impracticable. Different species required different capture methods; all methods took considerable time in the aggregate, and often the 'bag' was disappointingly below expectations for some species. To avoid upsetting the 'balance' we decided to keep seasonal slaughter as low as possible. We aimed at taking a weekly (including peak tick activity) series of adequate samples, the total not exceeding 10 % of population, in the case of species with sufficient individuals; and, at the other extreme, a single adequate sample near peak time, even if it meant taking the entire population, in the case of species with very low densities. In practice, the difficulties often enforced some degree of departure from this scheme. On the question of adequate sample size, see Milne (1943) and § 7 below.

Since we are concerned with the relations of wild fauna and sheep we obviously had to take wild animals only from the grazing area open to sheep. Thus, at Lordenshaws, we practically confined our trapping and shooting to the open heather-bracken grazing and the rough-grass fields. The only exceptions were for certain animals more or less confined to the spruce-fir wood (fenced against sheep), and the scrub (the thicker parts of which sheep do not much frequent). Some of these do come out to work the edges of the open grazing along with sheep; we shot samples of the commonest ones seen on bushes and ground near the wood and scrub.

Immediately after death we put all animals, except very large ones like fox and badger, individually into white paper bags, closed tightly with string, to be examined 24 hr. later. By then most of the ticks had left the body and were found on the paper, but we also searched diligently in hair, fur and feathers. We went carefully over large animals in the field. Since ticks begin to leave a dead body after some hours, we visited 'mouse' traps frequently; a few ticks on 'mice' may, nevertheless, have been lost. Ticks were stored in tubes of 70 % alcohol. We identified the ticks, but relied on the later confirmation of Mr E. Browning of the British Museum, for whose massive labours we are most grateful. The entire tick collections for 1943, 1944 and part of 1945 are lodged in the British Museum, with date, locality and number of individuals for each animal-species sample. In most cases the latter does not include the number of animal specimens in the sample having zero infestation.

Further details of methods are given throughout the paper, and the method for estimating host-species populations is dealt with in §§ 9 and 10. The Home Secretary gave permission (Statutory Rules and Orders, 1943) to kill wild birds contrary to the various laws and by-laws, on the understanding that the culpability (if any) of birds might be cleared up once and for all, that no indiscriminate slaughter would take place and that rare species would be spared.

In the body of this paper (Part 2) we use the common names for animals as given in the *List of British Vertebrates* (British Museum, 1935). In Appendix 1 we give the specific names corresponding to these common names, following the same list, together with the total number of specimens examined and some other details.

We showed (Part 1) that work of the present nature must be based on adequate samples at the right time. No previous author shows a proper appreciation of this point. Accordingly, in Appendix 2 we have given dates, sample sizes and tick counts for wild species, together with parallel observations on sheep sharing the same ground, in order that the reader can fully test our conclusions.

4. HOSTS TO *IXODES RICINUS* L.

During the investigation three other *Ixodes* species were found, namely: *I. hexagonus* Leach; *I. canisuga* Johnston; and *I. tenuirostris* Neumann*. Only mammals were involved. There were only six species, viz. dog, fox, otter, weasel, rabbit and short-tailed vole. However, for all hosts, including those six, *Ixodes ricinus* is incomparably the most important tick parasite in areas of north-east England where it occurs.

(i) *Hosts to one or more tick stages*

(1) Man, (2) horse, (3) cow, (4) sheep, (5) goat, (6) dog, (7) cat, (8) common mole, (9) common shrew, (10) hedgehog, (11) fox, (12) badger, (13) otter, (14) stoat, (15) rabbit, (16) brown hare, (17) short-tailed vole, (18) long-tailed field mouse, (19) red squirrel, (20) roe deer, (21) poultry, (22) carrion crow, (23) rook, (24) magpie, (25) starling, (26) chaffinch, (27) skylark, (28) meadow-pipit, (29) great titmouse, (30) willow-warbler, (31) song-thrush, (32) redwing, (33) blackbird, (34) robin, (35) whinchat, (36) wheatear, (37) hedge-sparrow, (38) long-eared owl, (39) kestrel, (40) common snipe, (41) lapwing, (42) pheasant, (43) common partridge, (44) black grouse, (45) red grouse.

The above list confirms previous British host records in twenty-one cases. Nos. 8, 9, 12, 13, 17, 18, 22, 24, 26–38, 40, 41 and 44 (i.e. twenty-four species) are new to the British host list, except that one or more of nos. 9, 17 and 18 may be covered by 'field mice', and no. 41 by 'green plover', terms used by Moore (see Part 1). The preponderance of small mammals and birds among the new-recorded host species confirms our impression (see Part 1) that previous workers tended to confine their attentions to the larger animals. The list of British mammal hosts has now increased from twenty-three to twenty-nine species and birds from twenty-one to thirty-nine (approx. totals). Moreover, Shcheglova (1939) mentions as hosts in Russia a further three mammals and four birds occurring in the British fauna, viz. bank vole, house mouse, yellow-necked mouse, tree-pipit, nuthatch, missel-thrush and great spotted woodpecker. This indicates that our British host list is still incomplete.

* A letter from Mr E. Browning (27/11/47) now states that '*Ixodes tenuirostris*, Neumann (1901) is synonymous with *I. triangulicep*s, Birula (1895)'.

(ii) *Non-hosts*

Appendix 1 shows that samples of thirteen mammal and bird species yielded no ticks. Nine of these are mentioned as hosts in Part 1, viz. weasel, bank vole, brown rat, jay, pied wagtail, sparrow-hawk, woodcock, curlew and wood-pigeon. Now Appendix 1 also shows that even the tick-yielding species have their uninfested individuals. Therefore, our failure to find ticks can be explained on grounds of sampling inadequacy (e.g. two weasels) and/or of habits (e.g. woodpigeon roost in the spruce-fir wood of Lordenshaws, but feed far afield on tick-free arable, seldom coming down on the infested Lordenshaws grazings). The same sort of reasoning could be extended to embrace the four remaining species samples for which there are no host records, viz. one lesser redpoll, one coal titmouse, one swift and three tawny owls. The swift perhaps skims the vegetation tips occasionally, but it would probably require a very large sample to produce a single infested specimen. But we need not pursue this aspect further because, judging from the length and diversity of our host list, we doubt very much whether any bird or mammal in tick-infested country could be absolutely a non-host; whether an animal's habits allow it more or less opportunity to meet ticks is another matter.

Below birds and mammals, however, the picture is different. We found no ticks on fourteen adders, four common frogs and three common toads. In Russia, Shcheglova (1939) found none on thirteen adders, sixteen common frogs, eight smooth newts, five *Rana terrestris* Andr. and ten *R. ridibunda* Pall. (his own nomenclature). On 5 June 1945 we placed two female and four nymphal *Ixodes ricinus*, taken from a blanket drag (i.e. ready to feed), on the backs of three live common frogs, the latter being in a glass jar suitably humid; the ticks had failed to attach by the end of 3 days. We conclude that no host occurs below the reptile level among vertebrates; and among British reptiles only the common lizard has been found a host—rarely.

Invertebrates are never mentioned in previous work (see Part 1). The inference, therefore, is that the hosts to *I. ricinus* are confined to vertebrates. Certainly we found no infestation among the large numbers of unwanted ground beetles caught in sunken canister traps set for voles and shrews. Nor have we seen ticks on humble bees.

(iii) *Hosts to the different tick stages*

(*a*) *Imagos*

Male ticks seldom attach themselves to any host. Ordinarily they get on a host merely to find a mate. Thus hosts to female ticks will necessarily be hosts to male ticks, and we shall refer only to the former here. Observations must naturally be confined to the female tick activity season, usually mid-March to the end of June in north-east England. All the animals listed in Appendix 1 were sampled in this period, most of them in sufficient numbers.

Farm and domestic mammals—horse, cow, sheep, goat, pig, dog, and cat, more especially sheep and cow—are habitual hosts to female ticks (see Part 1, and Milne, 1945*c*). Female ticks occasionally manage to attach to human beings, even when precautions are taken. No case of female infestation in poultry has ever been recorded. Perhaps this is merely because poultry are generally confined to the farmyard, a habitat unsuitable for ticks. At all events, the hen-like pheasant certainly becomes infested with female ticks when opportunity offers (see below).

In the available samples of northern England wild mammals we found female ticks on eight species only, viz. hedgehog, fox, badger, otter, stoat, rabbit, brown hare and roe deer. From Part 1, Table 1, we may add Scottish mountain hare and red deer (the single brown rat recorded in Part 1 is probably 'accidental'). Since no female ticks were found on mole, shrew, vole and field mouse, there is obviously a strong tendency for females to be confined to the larger mammals. If we include man and domestic and farm mammals, then nineteen of the twenty-nine mammals known to be tick hosts in Britain are hosts to female ticks (65·5 % of infested species).

Among thirty-nine host species of British birds, wild and domestic, only three—magpie, pheasant and red grouse—have yielded female ticks (7·7 % of infested species). As in mammals, the female-tick-infested birds are among the larger species.

Note. In comparison with mammals 7·7 % is too high since we have not subdivided 'poultry' into their several species as in the case of domestic and farm mammals.

(*b*) *Nymphs and larvae*

Data in Part 1 and in Appendix 2 of the present Part show that, of the sixty-eight species (approx.) of British mammal and bird hosts, forty-eight have yielded both nymphs and larvae. The remaining twenty species may be dealt with as follows.

Although given as hosts (Part 1) there are no details of stages infesting weasel and Scottish mountain hare; they cannot well differ from stoat and brown hare which are infested by both nymphs and larvae. On the only two otter specimens no search was made for nymphs or larvae but since all the larger mammals, including fox and stoat, are infested by both tick stages, there is no reason to doubt that the otter would not be similar. In the same way, although a solitary rat (Part 1) is recorded as having only a female tick, that animal should not differ from say short-tailed vole or rabbit if it comes in contact with tick-infested vegetation.

Samples of four species—badger, roe deer, redwing, and barn owl—yielded nymphs but no larvae. The very fact that they had nymphs, which are less numerous than larvae, should indicate inadequate sampling, i.e. that they must also be hosts to larvae. This idea is strengthened by allusion to other species which have yielded larvae: thus compare badger with fox and stoat; roe deer with red deer and sheep; redwing with song-thrush; and barn owl with long-eared owl.

Samples of thirteen species yielded larvae but no nymphs, namely, common shrew and common mole; long-tailed field mouse; jay; great titmouse; song-thrush; robin; siskin and whinchat; merlin; common snipe and golden plover; and black grouse. Again, with the exception of the first two, these can all be compared with similar sizes and types of animals which have yielded nymphs, e.g. short-tailed vole; magpie; chaffinch; meadow-pipit and blackbird; sparrowhawk; curlew and lapwing; and red grouse. Moles are largely uninfested (only two in seventeen specimens, Appendix 1); they have little contact with the vegetation and, therefore, if they encounter ticks at all it will most likely be the most numerous stage, namely, larvae. We shall deal with shrew below.

Adequate sampling at the right time is all-important in establishing whether an animal is a host at all, and still more so in showing how many tick stages are concerned. Thus previous authors (see Part 1, and Shcheglova, 1939) found certain animals uninfested which we found infested (e.g. skylark), and vice versa (e.g. bank vole); or they found an animal infested with fewer tick stages (e.g. red squirrel) and we, vice versa (e.g. common lizard). But our shrew sample is fully adequate from the viewpoint of both numbers (42) and times of capture (see Appendix 2). We have shown the approximate host-size level below which female infestation becomes very rare ('accidental'). If the exceptionally circumstanced mole is omitted, this level in the case of nymphs apparently excludes only the shrew, the smallest northern England host.

Considering all the above facts, we may conclude that: (1) all the recorded British hosts, except shrew and mole, are hosts in some degree to nymphs; and (2) all, without exception, are hosts to larvae.

(iv) *Differences in mammal and bird infestations*

Above, paragraph (iii *a*), shows that proportionately more mammal than bird species are hosts to female ticks in Britain. If proneness to infestation

with this stage is a function of size, as the confinement of females to the larger animals seems to suggest, then this could be due to the mammals having about a dozen host species larger than the largest bird host, the pheasant. In § 8, we suggest a reason for the relation between host size and female infestation. A further difference between birds and mammals is suggested in § 5.

5. SELF-DETICKING

In large mammals, e.g. cow and deer, ticks are practically never found on the back and upper sides; in small mammals, e.g. hare and vole, ticks are quite often found on these areas. This may be associated with some limit to the distance a tick requires to crawl over the body before becoming sufficiently stimulated to attach (see Lees in Milne, 1947*b*). After that, as a general rule, attached ticks are distributed all over the mammal body, although they usually decrease in numbers from head back to tail, especially in the larger mammals (cp. Milne, 1947*b*, for sheep). The rabbit is a curious exception. Nymphs and larvae were plentiful on chin, lips, nose, around eyes and on ears. But apart from one female tick on the scrotum of one individual, we never found a single tick (of any stage) farther back than the neck in 317 rabbits. This cannot well be put down to self-deticking, because the rabbit has a much clumsier mouth than the short-tailed vole which has ticks occurring all over its body, including the tail.

In the 320 bird specimens (see Appendix 1), on the other hand, we found all ticks confined without exception to the head and upper neck. We put this beyond doubt, immediately after shooting in many cases, by severing the neck at the highest point reachable by the beak and promptly bagging the two portions of the body separately. This finding points to universal active deticking by birds. It suggests also that the beak is more efficient than the teeth, even of small mice, for picking off engorged larvae.

Shcheglova (1939) says: 'Absence of adult ticks (at any rate females) on the majority of wild birds and mammals is possibly explained by adult ticks and to a lesser extent nymphs—particularly after they have fed—being more easily destroyed by the hosts themselves... by biting or pecking. This is supported by experiments made by N. I. Alfeev (1935), who observed a bank vole (*Evotomys glareolus*) in captivity rapidly devour female *Ixodes ricinus* that were put on it. On hedgehogs the ticks are completely secure in this respect, among the spines....'

Note. Shcheglova dealt only with the smaller mammals.

The larger mammals probably cannot deal effectively even with engorged female ticks, their mouths being too clumsy. (We do, however, occasionally find burst females on those parts of the body which can be rubbed against scratching points, i.e. posts, rocks, etc.) But we cannot imagine that any small animal with sharp teeth or fine-pointed beak will readily tolerate so bulky and irritating an excrescence as an engorging female or even nymphal tick within reach. Later, we shall propose an additional and probably more important reason for absence or scarcity of female ticks on the smaller animals (§ 8).

Self-deticking seems, therefore, to be practised by some animals, and may be a factor not only in determining their own host potential, but also in reducing the tick population of their common feeding ground. We shall deal with predators proper in a later paper.

6. PROPORTIONS OF TICK STAGES IN RELATION TO HOST SIZE

Although unfed female, nymphal and larval ticks are all active together in April–June, their relative activity varies (see Table 1, and Milne, 1945*a*, 1947*a*). Hence, for comparing proportions of tick stages on different hosts, sampling must be as nearly simultaneous as possible. In this, and other respects, Table 1 shows the best data available from Appendix 2, for animals spending a great deal of their time in contact with the ground vegetation where ticks are to be met.

Three size-classes are compared: (i) sheep, (ii) rabbits and large birds, (iii) 'mice' and small birds. 'Mice' and birds are listed by name in the heading to the table. The sheep were at Hethpool, the wild animals at Lordenshaws. Hethpool, only 21 miles north-north-west along the eastern edge of the Cheviot range, enjoys the same climate as Lordenshaws. Moreover, both farms are similar as to depth of vegetational cover. Accordingly, we may reasonably assume that the proportions of tick stages in their ground populations should be roughly the same in any one year. That is, in Table 1, the sheep data should be comparable with that for the wild animals, even though from different farms.

In practically every instance we may calculate from Table 1 that the smaller the host the less the females/nymphs ratio and, similarly, the less the nymphs/larvae ratio. Table 2, constructed from 'totals' in Table 1, exemplifies this fact in average form. Ratios calculable from the totals of the best block of data (best with respect to sample sizes throughout the host series), namely, the data for 18, 21 and 24, 28 April, show the same thing. In view of variation, the ratios in Table 2 must not be taken as very precise in themselves, but the differences are so large and the picture so similar in both animal series that we cannot avoid the general conclusion: the smaller the host, the fewer the females relative to nymphs and the fewer the nymphs relative to larvae.

Discussion. § 5 suggests that, in general, deticking is probably practised more efficiently the smaller the host and the larger the tick stage. The observed differences in proportions of tick stages relative to host size might, therefore, be thought to be due in some measure to deticking. But the differences between large and small birds are of the inaccessible to the beak. Hence we may dismiss deticking as no more than a minor factor at best, and that only in the mammals, so far as the proportions in Table 2 are concerned. The differences in proportions must, therefore, be mainly a reflexion of the frequencies with which the various tick stages are encountered by the different sized hosts. Now the

Table 1. *Average tick counts from samples of sheep at Hethpool, and rabbits, 'mice', large birds and small birds at Lordenshaws, in* 1944

'Mice' include common shrew, short-tailed vole and long-tailed field mouse; large birds: pheasant, partridge, red and black grouse; small birds: skylark and meadow-pipit. The first date applies to Hethpool, the second to Lordenshaws, e.g. 11, 14 April means 11 April at Hethpool and 14 April at Lordenshaws. N = number of individuals per sample; ♀♀ = female ticks; oo = nymphs; LL = larvae. Hogg ('i.e. one-year-old sheep') tick counts are employed instead of the ewe counts in Appendix 2, because the hogg dates correspond more closely with the wild animal dates. Sheep counts are 'standard', i.e. for the fore end of the body only, while wild animal counts are 'total', i.e. for the entire body (see Milne, 1943, 1947*b*).

Note. 1944 was a year in which nymphal peak was well behind female peak activity.

Farm	Animal	Item	11, 14 April	18, 21 April	24, 28 April	1, 5 May	8, 12 May	15, 19 May	23, 26 May	29 May, 2 June	13, 16 June	April–June Totals
Hethpool	Sheep	N	10	10	10	10	10	10	10	10	10	90
		♀♀	4·6	9·6	10·7	18·6	15·2	10·5	14·1	9·9	6·9	100·1
		oo	52·8	37·2	36·3	102·6	91·9	80·1	114·1	87·7	116·8	719·5
Lordenshaws	Rabbits	N	10	10	12	10	13	10	10	10	10	95
		♀♀	0·10	0·00	0·08	0·10	0·00	0·00	0·00	0·00	0·00	0·28
		oo	2·60	1·70	2·92	4·70	1·77	2·40	1·90	0·50	1·10	19·59
		LL	0·30	1·90	1·42	7·40	7·23	12·00	18·90	9·40	10·10	68·65
Lordenshaws	'Mice'	N	1	3	15	2	2	11	2	2	3	41
		♀♀	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
		oo	0·00	0·33	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·33
		LL	2·00	0·67	5·53	0·50	2·00	0·45	2·00	4·00	0·67	17·82
Lordenshaws	Large birds	N	—	5	8	—	—	1	—	1	1	16
		♀♀	—	0·40	0·25	—	—	0·00	—	0·00	0·00	0·65
		oo	—	2·40	9·50	—	—	0·00	—	15·00	2·00	28·90
		LL	—	0·80	2·63	—	—	1·00	—	36·00	3·00	43·43
Lordenshaws	Small birds	N	—	10	5	—	—	5	—	9	9	38
		♀♀	—	0·00	0·00	—	—	0·00	—	0·00	0·00	0·00
		oo	—	0·10	0·20	—	—	0·00	—	0·64	0·33	1·27
		LL	—	0·10	0·00	—	—	0·40	—	13·33	11·89	25·72

Table 2. *Ratios* (× 100) *of females to nymphs, and nymphs to larvae, on different sizes or size groups of host species* (*calculated from 'totals' in Table* 1)

Hosts	Ratios: Females/nymphs	Ratios: Nymphs/larvae
(i) Sheep	13·9	Larvae not counted
(ii) Rabbits	1·4	28·5
(iii) 'Mice'	0·0	1·8
(i) Sheep	13·9	Larvae not counted
(ii) Large birds	2·2	66·5
(iii) Small birds	0·0	4·9

same order as those between rabbits and 'mice' (see Table 2). And we know that deticking can have played no part in the case of the birds, since counts are from head and upper neck only, i.e. regions

proportions of tick stages on the ground are fixed for any one time. Hence, there must be different degrees of availability of the various tick stages to different hosts. Otherwise, all hosts in simultaneous samples would exhibit the same proportions in attached stages. Shcheglova (1939) suggests that larvae are active at a lower level in the vegetation than other tick stages. Tambs-Lyche (1943) gives some data indicating that, when the vegetation from ground level (grass) to bush tips (juniper and alder, up to 1+ metres high) is sampled, nymphs appear to be active at a higher level than larvae, and females higher than nymphs. This would appear to explain why the smaller (less tall) a host, the less the proportion of females to nymphs met, and similarly the less the proportion of nymphs to larvae. Unfortunately, our own observations (unpublished) reveal

that the great majority of individuals of all tick stages on bush-free pasture (as 97 % of the typical hill farm here, see § 2) are active at the same level, i.e. at or near the tips of grasses, rushes, etc. In short, we must accept the facts of Tables 1 and 2 without being able to explain them.

Before leaving this section we should point out two expected facts obvious in Table 1. First, larvae are seasonally more numerous than nymphs on hosts, and nymphs more numerous than females. Secondly, even though sheep counts are not 'total', there is a relation between weight of infestation and host size; this will be dealt with in § 8.

7. VARIATION IN INDIVIDUAL WEIGHT OF INFESTATION WITHIN A SPECIES

We showed (Milne, 1943) the very wide variation in weight of female tick infestation among sheep individuals at the same time and from the same place. For example, during high activity ten sheep on one section of a grazing showed a range of 12–243 female ticks per sheep. Female ticks are comparatively few on the wild fauna (except red deer), but we now have masses of figures indicating the universality of high infestation variation in different species. The following selections from high activity periods, each animal sample being comprised of individuals from the same place at the same time, illustrate the point:

		Ticks
Female ticks:	3 roe deer	1–14
	2 brown hares	1 and 12
	10 brown hares	0–6
Nymphs:	10 sheep	28–270
	10 rabbits	0–31
Larvae:	10 rabbits	0–82
	2 short-tailed voles	9 and 98
	6 meadow-pipits	6–23

(*Note.* Different animal species were not sampled at the same time, so these data do not reflect infestation weight in relation to host size.)

With such high individual variation, the standard error of the mean will be comparatively large, and hence samples should always be as big as possible in comparing infestation weights of species.

8. SIZE OF HOST IN RELATION TO WEIGHT OF INFESTATION

Presence as opposed to absence of ticks is a crude measure of infestation weight. On the relation between the latter and host size, the first broad indication, therefore, is the fact that female ticks are confined to the larger animal species (§ 4). A less coarse indication emerges from consideration of relative numbers of infested and uninfested individuals. Appendix 1 shows that a proportion of individuals in many host species are uninfested. This proportion is lower, of course, the nearer peak activity time. But some species have a higher proportion than others. The 'best block of data' in Table 1 (18, 21 and 24, 28 April 1944, already referred to in § 6) shows the following distribution of individuals with respect to presence or absence of females and nymphs:

	♀♀		♂♂	
	with	without	with	without
20 sheep	20	0	20	0
22 rabbits and 13 large birds	5	30	25	10
18 'mice' and 15 small birds	0	33	3	30

(In the case of larvae: no data for sheep; and no statistically significant difference between the other two animal groups.)

A large part of the data in Appendix 2 can be used in rougher fashion to give much larger groups, and the results are similar. Clearly the proportion of infested becomes less with decreasing body size. No detailed statistical test is necessary to confirm that.

We come now to the precise measure of infestation weight, namely, actual numbers of ticks. At peak activity, on long-established heavily infested ground, average individual female tick infestations for certain different (sizes of) mammals are approximately as follows: red deer, 500; sheep, 150; brown hare, *c.* 10; rabbit, less than 1; and 'mouse', 0. These estimations are based on Appendix 2 and unpublished data, Milne (1947*b*), and Part 1 of this paper. Similar descending ranges at higher levels obtain with nymphs and larvae. And the picture is the same in birds as in mammals. In the present connexion the sheep counts of Table 1 are not strictly comparable with those for the wild animals, since the 'total' count for ewes would be about half as big again (see Milne, 1947*b*). But even without this adjustment the table clearly shows that, for the particular animals concerned, the smaller the host the less its weight of infestation in the case of any tick stage.

Discussion. Even a very heavily infested pasture yielded no more than about twenty unfed females and round about 1000 unfed nymphs per 100 sq. yd. over an entire year (Milne, 1946). Only a small fraction of these numbers is available in any short period such as represented by our counts. In short, active unfed nymphs and more so unfed females are relatively very sparsely distributed in nature. Therefore, the animal covering most ground (in contact with the appropriate vegetation, of course) will pick up most ticks. The smaller the animal the less the breadth of front (i.e. of body) presented and generally the less the linear distance travelled in search of food, etc.; that is, the less the area of ground swept in unit time the less the chances of

encountering ticks (Milne, 1947*b*). This seems to be a reasonable explanation of our finding that the smaller the host the less the weight of infestation. That finding is true in general, but in detail there are expectedly numbers of exceptions. Habits are not set to one pattern. Some smaller animals are more active in their peregrinations; others come more in contact with the appropriate vegetation. Thus, on Lordenshaws, the highest individual female tick count among hundreds of rabbits was 5 as against 8 in only five stoats; the stoat is the smaller but covers much more ground. Again, the mole is less infested than the smaller shrew (see Appendix 2); but the mole, living more underground, has less opportunity of meeting ticks. There are other examples in Table 4, but we need not labour the point. The conclusion should be: the smaller the host, generally the less the weight of infestation.

9. HOST POTENTIALS FOR FEMALE TICKS ON FIVE TYPICAL NORTHERN ENGLAND HILL FARMS

(i) *Methods*

The comparative host potential of a species is simply an estimate of the total ticks which could be fed by that species in a season compared with a similar estimate for a standard species going on the same ground. Obviously, species host potential is the product of (1) the average seasonal tick total for the individual, and (2) the number of individuals present.

(1) In § 3 we showed that the scale of sampling necessary for direct calculation of comparative seasonal tick totals is out of the question for many wild species. The alternative was to assume that average tick counts at any moment bear the same relation to each other as average seasonal totals. Since different species on the same grazing exhibit tick activity curves similar in form and coinciding in time (Milne, 1947*a*), this seems a perfectly reasonable assumption, provided that, in view of variation (§ 6), the necessity for adequate samples and repetition is kept well in mind.

(2) Although the position with regard to British birds has improved within recent years, published information on population densities for even the commonest British wild mammals is conspicuously lacking. To study the population dynamics of one species alone, and in one place only, is no mean task (cp. the team-work of the Bureau of Animal Population, Oxford, on voles, for instance). Having the entire host faunas of several localities to contend with, we had no option but to rely, for the most part, on the estimates of gamekeepers, trappers and shepherds. These estimates tended to be springtime average adult figures, but birth- and death-rates, in relation to the timing of the tick activity curve, must influence host potentials to some extent. Precise information on birth-rates is scanty enough, but, on death-rates, almost totally lacking.

Such is a brief outline of the difficulties. Later we shall return appropriately to several in essays at rationalizing them. But clearly it would be an immense task to collect sufficient data to provide very precise comparative host potentials. Accordingly, conscious of the shortcomings of our data (Appendix 2), we attempt no more than broad outlines. This has been done in greatest detail for female ticks (present section), because by design (see § 3) our data are best for this stage; but indications are given for the other stages (§ 10). The method consists in calculating the sheep equivalent of each species with respect to ticks, i.e. using the sheep as the standard.

(ii) *Sheep equivalent of the average adult individual among the wild host species*

Complying with the required conditions of time and place, Table 3 shows comparable tick counts for sheep and ten of the British wild hosts. In the case of the wild animals, counts for single individuals are given; in sheep, the mean count for eight to ten individuals.

Although twenty-five foxes were examined (Appendix 1), only nine have comparable sheep counts available. The samples for six animals appear rather small (less than ten), but this was not due to lack of effort in seeking them. In three of these—fox, badger and roe deer—the sample is actually larger than the total population on the average northern England hill farm; in the otter it is equal to this population; and in hedgehog and stoat about one-half and one-third respectively; and to comparative rarity was added differing degrees of difficulty in effecting capture. In some cases the individuals in a species sample necessarily came from different farms; but the individuals are always compared with sheep going on the same ground from which they came.

In two cases, badger and stoat, the distribution of tick counts in our samples appears rather curious at first sight. In each case there is one individual with a relatively high infestation, while the remaining individuals are uninfested. In view of the high variation among individuals (§ 7) this is understandable, however, on the inference that the infested individual was highly susceptible to infestation, while some of the uninfested were the opposite (see also Milne, 1947*b*). Remembering that, with a mean of 85·7, ten sheep can have a range of 12–243 ticks, the amount of positive correlation, weak though it is, in each animal series shown in Table 3 is surprising. We see a distinct (and expected) tendency for the wild animal counts to follow the sheep counts at a lower level.

Because of their unwieldiness we have not included the data for rabbit in Table 3. About ten rabbits were compared weekly with about ten sheep from the same grazing. For thirty-one occasions (weeks) the totals were 294 rabbits and 287 sheep. The individual sheep counts varied, as illustrated in a previous paper (Milne, 1943). Of the 294 rabbits only eight yielded female ticks, the individual counts being: 1, 1, 1, 1, 1, 2, 2 and 5. The mean for rabbits was 0·0476 tick and for sheep 34·57. The final column of Table 3 gives similar general means for the other wild animals.

level. This would be quite simple if the raw data (Table 3) were distributed normally. Unfortunately, they are not so. The curious distributions are understandable when we think of the seasonal tick activity curve (see Milne, 1945*a*, 1947*a*), and the fact that for each species the individuals comprising the total sample were (necessarily) taken at different times during the season. Had the individuals been taken all in one day (in the case of each animal) then the tick count distributions would have approached normality sufficiently for practical purposes (see Milne, 1943). The question, therefore, is: since direct

Table 3. *Female tick counts on individual wild animals compared with mean female tick counts for groups of eight to ten sheep running on the same ground at the same time*

Animals	Ticks on individual wild animals and mean ticks on groups of eight to ten sheep	General means‡
5 hedgehogs	3 1 1 0 0	1·000
49 sheep	19 11 1 6 1	7·388
9 foxes	4 4 3 2 1 0 0 0 0	1·556
65 sheep	169 128 10 109 9 25 9 1	67·11
7 badgers	13 0 0 0 0 0 0	1·856
59 sheep	109 77 14 1 1 1	34·13
2 otters	7 0	3·500
18 sheep	26 169	108·1
5 stoats	8 0 0 0 0	1·600
46 sheep	74 36 25 3 1	29·23
11 brown hares	12 1 11 5 0 4 4 4 0 0 0	3·727
84 sheep	75 57 85 119 33 9 24 1 1	46·33
8 roe deer	14 4 1 11 4 3 3 2	5·250
60 sheep	41 91 40 20 14 19	37·60
11 magpies	1 0 0 0 0 0 0 0 0 0	0·091
87 sheep	174 109 85 77 36 33 16 9 8	60·71
11 pheasants	4* 1 1 1 0 0 1 0 0† 0 0	0·727
76 sheep	77 85 36 8 29 27 19 9	36·03
18 red grouse	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0·056
100 sheep	85 109 67 36 20 19 4 2 1 1 1	32·23

* Sick, therefore abnormally heavily infested. † Sitting on eggs, therefore not likely to be infested.
‡ In case of sheep, calculated on total individuals involved, not on the given group means.

From Table 3 and the above rabbit data we have calculated the sheep equivalent of the average individual, of the eleven northern England wild hosts, with respect to female ticks. These sheep equivalents are merely the ratios of the actual general means, ignoring sampling error. Thus, for one hedgehog, the sheep equivalent is 1·000/7·388 = 0·1353 of a sheep. The ratios are gathered, in descending rank, in Table 4. It would be extremely useful if we could calculate the fiducial limits of these ratios (sheep equivalents), i.e. the limits within which the true ratio will lie at a given probability

statistical treatment is impossible, how are we to judge the reliability of the ratios in Table 4? Table 3 gives data for seven single brown hares and two pairs taken on nine different occasions. Here the average hare was equivalent to 0·081 of a sheep. Now at Whitfield, in 1946, we had an orthodox sample: ten hares and ten sheep tick-counted on the same day. The female counts for individuals were, hares: 0, 0, 1, 2, 3, 4, 4, 4, 5 and 6; sheep, 11, 24, 24, 34, 35, 42, 45, 52, 62 and 67. The mean for hares is then $2·90 \pm 0·66$, and for sheep $39·60 \pm 5·58$, giving a ratio of 0·073, comparing well with 0·081 above.

Admittedly this is only one instance, but it rather suggests that the data in Table 3 may be as reliable as those from orthodox samples of the same size. If this is so, the fiducial limits of the ratio derived from the orthodox sample should give us a rough idea of the order of error attached to the ratios listed in Table 4. For the working formula in this instance we are indebted to Mr D. J. Finney, Oxford.

Table 4. *Sheep equivalents for the average adult individual of different species with respect to female ticks (ignoring sampling errors)*

The species comprise the known wild hosts to female ticks on northern England hill and moorland farms.

Species	Sheep equivalent
Roe deer	0·1396
Hedgehog	0·1353
Brown hare	0·0771*
Stoat	0·0547
Badger	0·0544
Otter	0·0324
Fox	0·0233
Pheasant	0·0202
Red grouse	0·0017
Magpie	0·0015
Rabbit	0·0014

* Whitfield data as well as Table 3 data included—see text.

Finney says: 'Fisher has referred to this problem in section 26·2 of the new tenth edition of his *Statistical Methods for Research Workers* (see also Fieller, *Quart. J. Pharmacy*, **17**, 117). The fiducial limits are given by

$$\frac{m}{1-g} \pm \frac{t}{b\,(1-g)}\sqrt{[s_1^2\,(1-g)+(ms_2)^2]},$$

where $m = a/b$, $g = (s_2\,t/b)^2$, a and b are the means for wild animal and sheep respectively, s_1 and s_2 the standard errors of these means (each based on $n-1$ degrees of freedom), the value of t being read for $n-1$ degrees of freedom and the chosen (5 %) level of probability.' In the case of the Whitfield hares

$$a = 2{\cdot}90, \quad b = 39{\cdot}60, \quad s_1 = 0{\cdot}66, \quad s_2 = 5{\cdot}58,$$

and for 9 degrees of freedom $t = 2{\cdot}26$. From this we calculate that, although on actual means one hare is equivalent to 0·0732 of a sheep, the true equivalence value lies somewhere between 0·0340 and 0·1288 on the 5 % probability. Now, the comparative consistency of the data in Table 3; the fact that samples of some species are equal to, or bigger than, their populations on the average farm, while samples of the remainder are relatively large; and the fact that the true equivalent for hare is unlikely to be greater than twice, and might equally well be half, the observed equivalent; all this suggests that if we multiply Table 4 equivalents by four there should be little danger of underestimation.

Table 4 shows that the average roe deer, hedgehog and brown hare have the highest sheep-equivalents; rabbit, red grouse and magpie the lowest; while stoat, badger, otter, fox and pheasant are intermediate. The data do not merit any finer grouping. Even the 'best' wild animal, the roe deer, is far behind the sheep as a host individual. The finding for red grouse, on a better sample, confirms Moore (see Part 1). The equivalent for brown hare suggests that his 'large numbers' of female ticks on Scottish mountain hare may be an exaggeration, unless the latter animal has some peculiar susceptibility. The very low infestations for roe deer compare badly with the high ones for red deer, even allowing for size (see § 8 and Part 1). The explanation must lie in habits. Red deer frequent the open grazings, while roe deer haunt the woods and better, less tick-infested, 'inbye' land on farms. The fox has a surprisingly low equivalent, borne out by an additional sixteen specimens not in Table 3 (see Appendices 1 and 2); we can hazard no reasonable explanation.

Note. Considering the nature of the data, sheep equivalents may seem to be calculated to an extravagant number of decimal places; this is merely for the sake of accurate arithmetic in subsequent calculations.

(iii) *Populations of adult hosts*

As mentioned before, there is, in British literature, little or nothing to serve as a guide on the question of wild mammal population densities on hill and moorland. Elton (1942) gives an account of the British work on 'mice', but this is too narrow in scope to afford very much help; in any case, mice, voles and shrews are hosts only to immature tick stages. His non-quantitative descriptions (1937) of relative abundance of hedgehogs in lowland country are also of little value here. The position with regard to wild birds is rather better, though still inadequate. Lovat (1911) gives firm figures for grouse density with useful generalizations on British moors. Among the modern bird censuses, the most useful is one by Lack (1935). This author gives the 'breeding bird population of British heaths and moorland' from a study of sixteen regions in Great Britain and Ireland. Apart from these, useful only for general confirmation, we have had to rely almost entirely on estimates by gamekeepers for our five typical farms. The good gamekeeper makes it his business to assess the populations of game and vermin on his land. All of the eleven northern England hosts to female ticks come into these two categories. The gamekeepers' estimates were invariably confirmed by independent accounts from shepherds and trappers. After 4 years of close contact with the men and their methods, the animals and the land, and after checking wherever possible (see notes following) we concluded that,

with the possible exception of the hedgehog, the estimates are fairly reliable.* (In any case, underestimation would require to be tremendously gross indeed, i.e. very much greater than we would reasonably expect, before the general conclusions in (v) below would be materially affected.) Table 5 shows these estimates, together with sheep and cattle populations. Of course wild populations vary from year to year, but the figures given are average. The sheep and cattle populations are kept steady. The following are a few notes on the estimates in Table 5.

The hedgehog is apparently not common on northern England hill and moorland grazings. Although upwards of twenty people kept a sharp lookout on the five farms for periods ranging from 1–4 years, only six specimens were taken in all, one unfortunately when female ticks were inactive. The hedgehog prefers 'white' (i.e. not heather) land with plenty of hedgerows and wood-edges for retreat. The latter are scarce on hill farms. Hence probably the animal's rarity. On the heathery, poor, exposed land of Lordenshaws, the gamekeeper, shepherd and writer searched diligently over 4 years without finding a single hedgehog. We concluded that they must be very rare if not absent altogether.* On the grassy Hethpool, an average of seven hedgehogs were taken in vermin traps each spring for several years on the edges of one conifer 'shelter belt'. The estimate of thirty-five is based on there being four other similar shelter belts on the farm. This estimate accords well with the findings on a neighbouring stretch of land similar in type and area (1000 acres). Here, for years, the gamekeeper did his best to exterminate hedgehogs: his diary shows an average of thirty-five killed per annum. The same man is now at Whitfield so the estimates for Parkhead and Mainsrigg should be similarly reliable. The Crag estimate is by the farmer, a singularly good observer of wild-life; it may be an underestimate since he is very cautious. Indeed all the hedgehog data may be slightly underestimated. Because a man pursues hedgehogs with intent to exterminate does not necessarily mean that he manages to kill them all, though he may come fairly near that.

Table 5. *Estimated populations of adult female tick hosts on five typical northern England hill farms*

Farm	Lordenshaws	Hethpool*	Parkhead	Mainsrigg	Crag	Means†
Acreage	1285	1124	1493	1639	2000	1500
Heather (%)	96	0	66	66	20	—
Hedgehog	0	35	6	8	12	12
Fox	3	6	3	3	4	4
Badger	2	4	2	2	2	2
Otter	0	2	2	2	2	2
Stoat	10	40	6	10	12	16
Rabbit	1200	2000	100	60	100	692
Brown hare	10	20	20	40	8	20
Roe deer	2	0	8	2	4	3
Magpie	8	0	2	2	4	3
Pheasant	20	20	50	100	20	42
Red grouse	200	0	800	600	12	322
Sheep	400	1000	450	600	500	590
Cattle	6	40	25	50	30	30

* Plus 24 'wild' goats visiting a section of the farm.
† To nearest whole number.

As we suggested above, the estimates for the other ten wild animals are reasonably acceptable.

The sites of fox dens and badger earths are always known on hill farms because both animals steal lambs, the former, of course, being by far the greater culprit. Usually there is one, and not more than two, dens or earths on the 1000–1500-acre hill farm. The fox ranges fairly widely, probably including two or three farms in his hunting, but the badger is less of a wanderer. A pair of badgers in hill and moorland country are reckoned to require at least 500 acres for their upkeep.

The otter is a very wide ranger, moving swiftly between estuary and watershed during the year. He must be of very little importance in maintaining the

* On reading §§ 9 and 10, Mr Charles Elton, Director, Bureau of Animal Population, Oxford, commented: 'I would not ordinarily accept keepers' estimates of density of animals, because they think in terms of the numbers of vermin killed, not the numbers remaining. But I think you have met this criticism quite reasonably by pointing out that the species concerned are either conspicuous forms, or else had been controlled almost to extinction. I am less happy about the vole density figures (see § 10) which are rather speculative; but for the purpose in view they seem to be a reasonable sort of guess, at any rate in determining the general scale of importance' (in the host potential problem).

* Later, the body of one, killed by a vehicle, was found on the main road near one of the areas of scrub woodland.

ticks on any one farm because of his scarcity, the shortness of his visits, which may not coincide with tick activity, and because he spends much of his time in the water. For instance, although two each are recorded for Parkhead and Mainsrigg, the gamekeeper was convinced they were one pair moving along the streams far and wide. None have ever been seen on Lordenshaws probably because, being the watershed for the area, the streams are small, containing none or only a few small trout.

The independent estimate of forty stoats for Hethpool is confirmed from the same source as the hedgehog data. On the neighbouring similar area, the keeper, bent on extermination, killed seventy-four stoats in his first year and an average of forty per year for the ensuing 8 years. Naturally there would be infiltration, since the stoat is a fairly wide ranger. This particular keeper, highly skilled and experienced, put hedgehogs and stoats on really rough hill moorland at about one-quarter of the Hethpool figures. The data for the other four farms confirm him.

The rabbit data are amply confirmed by trapping accounts on all five farms. With respect to female ticks the figures are probably a little too high, because a proportion of the rabbits will be born towards the end or after the spring tick season, while trapping takes place in autumn and winter. Hethpool and Lordenshaws have quite high rabbit populations; Parkhead, Mainsrigg and Crag farm very low. The latter farms are not so well drained as the former and rabbits do not like wettish land. They like a sandy soil and good feeding. Lordenshaws is on sandstone, while Hethpool has sand only along the river; Hethpool, however, has by far the richer grazing.

We confirmed the brown hare data for Lordenshaws, seeing nine during a beat of the greater part of the farm in April 1946. On the heathery farms they lie up during the day and go down to the grasslands in the evening to feed.

The presence of roe deer is dependent on the amount of woodland cover within reach. Probably the narrow shelter belts at Hethpool are too small; at any rate roe deer have not been seen there in the memory of a 60-year-old trapper. The best conditions for roe deer are at Parkhead, with its 100 acres of bordering woods together with other woods at no great distance.

Magpies were not specifically kept down on Lordenshaws during the absence of the regular keeper on war service. As a rule they are systematically shot so that one or two pairs per 1000 acres is their usual maximum, as figures for the other farms show. The trapper mentioned in the preceding paragraph has never seen magpie on Hethpool, though he has seen both magpie and roe deer on the next farm lower down.

Pheasants, like roe deer, are dependent on woodland shelter. This is best on Parkhead and Mainsrigg, where, as the largish figures would suggest, they are also preserved.

The red grouse figures show that our selection of farms is a good cross-section of hill and moorland types in northern England. Parkhead and Mainsrigg, two-thirds 'black', i.e. heather-covered, land of good quality, have grouse densities among the highest in Britain. Lordenshaws, almost all heather, but poorer and so well drained (sandstone) that young grouse die of thirst in a droughty nesting season, is among the poorer types of grouse moors. Crag with comparatively little heather (and that of poor quality), and Hethpool with none, are unsuitable for grouse.

On Hethpool (only) there is one other heavily infested mammal, the 'wild' goat. A herd of about twenty-four visits one section of the grazing from time to time through the year. These goats, domesticated at one time, now roam the eastern end of the Cheviots in two or three similar herds. On several occasions we examined superficially several nannies and kids; they were just as heavily infested with female ticks as the ewes and lambs on the grazing, if not more so.

Table 5 shows adult populations only. Adult sheep include all those from 1 year old upwards; adult cattle from 2 years of age.

(iv) *Sheep equivalents of the adult populations of the different wild hosts*

In Table 6 the animals are arranged in the order (rank) of their mean population sheep equivalents for the five typical farms. The calculations are made from the data in Tables 4 and 5. For the moment, sampling error in tick counts is ignored. Naturally, the order (rank) in Table 6 differs from that in Table 4 since populations of the various animals differ. Shcheglova (1939) also rates the hedgehog population highest with respect to female ticks but there were apparently no deer, hares, rabbits nor stoats on his pastures, and his biggest mammal was the polecat.

Our values for hedgehog and otter must undoubtedly be too high on the implied annual basis. Hedgehogs are usually not active until May, when half or more of the (spring) seasonal activity of female ticks is over; compare the consistently low tick count means for sheep at the times when hedgehogs were caught (see Table 3). The timing of the otter's short visits on a farm may, or may not, coincide with the season of tick activity (see § 9, iii). In short, these two animals are not available as hosts during the entire tick season on any northern England farm. Again, few of the wild female tick hosts will recognize the boundaries of a farm, especially wide-rangers like fox, hare, stoat and roe

deer. This may lead to slight overestimation of their population sheep equivalents for a particular farm, but on the whole will balance out from farm to farm.

(v) *The proportions of the female tick ground population supported by adult wild fauna and adult farm stock*

There is the possibility of two further female tick hosts in northern England, namely, common partridge and black grouse. We found no female ticks on either, but our samples were inadequate—only three specimens of each. From habit and size considerations, these birds could have average individual infestation weights no higher than red grouse and pheasant, and populations on the five farms ranged from six to twenty-four partridges and from nought to ten black grouse, i.e. both low. Hence the populations of these two birds would do very little to swell the total host potential of the entire wild fauna. In any case, several sources of slight overestimation have been mentioned in (iv) above. The chance of an occasional female tick on animals smaller than those listed as hosts to this stage is so trifling as not to be worth consideration.

goats. For example, the total wild fauna of Lordenshaws is equivalent to 4·21 sheep with respect to female ticks, and the farm carries 400 sheep; hence the adult wild hosts maintain $4{\cdot}21 \times 100/404{\cdot}2 = 1{\cdot}04$ % of the female tick population. For the five farms the range of this percentage is small and its level low, viz. 0·8–1·4 %. This is still true when we allow generously for sampling error, since the range is then, at most, only 3·2–5·6 % (i.e. multiplying values by four, as suggested in § 9, ii, above). In addition, numbers of cattle, a good deal more heavily infested than sheep (qualitative observation), besides a few horses, dogs, etc. on all farms, and goats on Hethpool, will lessen the proportion of female ticks fed by adult wild hosts on a farm grazing.

Table 6. *Population sheep equivalents, with respect to female ticks, for adult wild hosts on five typical northern England hill sheep farms*

Showing sheep equivalent for the adult population of each wild host on each farm, and sheep equivalent for total wild hosts; and hence the percentage of female ticks, on a grazing, supported by wild hosts. These values are calculated from Tables 4 and 5. The means suggest average conditions on northern England hill farms.

Species	Lordenshaws	Hethpool	Parkhead	Mainsrigg	Crag	Means*
Hedgehog	0·00	4·74	0·81	1·08	1·62	1·62
Brown hare	0·77	1·54	1·54	3·08	0·62	1·54
Rabbit	1·68	2·80	0·14	0·08	0·14	0·97
Stoat	0·55	2·19	0·33	0·55	0·66	0·87
Pheasant	0·40	0·40	1·01	2·02	0·40	0·85
Red grouse	0·34	0·00	1·36	1·02	0·02	0·55
Roe deer	0·28	0·00	1·12	0·28	0·56	0·42
Badger	0·11	0·22	0·11	0·11	0·11	0·11
Fox	0·07	0·14	0·07	0·07	0·09	0·09
Otter	0·00	0·06	0·06	0·06	0·06	0·06
Magpie	0·01	0·00	0·00	0·00	0·01	0·01
Total	4·21	12·09	6·55	8·35	4·29	7·09
Percentage of total female tick population maintained by wild fauna on farm	1·04	1·20	1·43	1·37	0·85	1·19

* Calculated from Table 4 and last column of Table 5.

In the second last row, Table 6 gives the sheep equivalent of the total adult individuals of the wild fauna; whence, using sheep populations in Table 5, the last row gives the percentage, of the ground population of female ticks on each farm, fed by the total adult wild fauna, assuming for the moment that no farm carries cattle and that Hethpool has no

As pointed out in para. (iii) of the present section it is extremely unlikely that the error in estimating the animal populations could materially alter the proportions. Therefore, to put 94–99 % of female ticks fed by adult hosts on a northern England farm as fed by farm animals, chiefly sheep, seems a safe estimate, and probably the higher figure is nearer the truth. The practical significance of this finding will be dealt with later (§ 11). Meanwhile, without comment at present, it is perhaps worth pointing out the remarkable similarity of the percentage results for all five farms.

(vi) *Discussion on the part played by the young of hosts*

Except for lambs (Milne, 1947*b*), we have no adequate tick counts for the young of hosts. Among

other things, the part played by young will depend on their size, number, and the time at which they come into the tick picture.

Usually in northern England, spring female tick activity commences sometime in March, reaches its peak in April or May, and ends sometime in June (Milne, 1945*a*, 1947*a*). There is little or no autumn recrudescence in the east; but in the west, from one-third to one-half of the spring weight of activity takes place with peak usually in September (Milne, 1945*a*).

The hill ewe generally has one lamb, born between 12 April and 10 May; a small proportion of ewes has twins. The relevant points about wild host breeding are as follows:

Hedgehog: about four young born not before June; sometimes a second litter in the autumn.

Fox: four to six young produced in April; one litter per annum.

Badger: three to four young produced in summer; one litter per annum.

Otter: two to five born any time of year, usually winter or early spring; one litter per annum.

Stoat: two to twelve, usually five, young arriving April or May; one litter per annum.

Rabbit: several litters of four to eight born summer and winter.

Brown hare: probably rather fewer litters (than rabbit) of two to five young born January–November.

Roe deer: one to two fawns arriving early in May; one family per annum.

Magpie: five to seven young, not ready to leave nest until about June; one brood per annum.

Pheasant: seven to thirteen young going about by early May; one brood per annum.

Red grouse: six to ten young going about by middle to end of May; one brood per annum.

These data for northern England, culled from gamekeepers and checked in the *Natural Histories*, are average. There seems to be some doubt about the otter, but for reasons given before, this animal may well be left out of our calculations.

Let us examine first the state of affairs in the spring season. Obviously the young of hedgehog, badger and magpie come into the picture too late to contribute anything. Before June the young of stoat, pheasant and red grouse will be too small to be hosts for female ticks (see §§ 4, 6 and 8)—even the (average) adult has a very low infestation weight (Table 4). The same applies to rabbit because, although their young begin to appear early, the adult has the lowest average infestation weight of all (Table 4), and, in any case, we have already made some allowance for young by using *autumn and winter* population figures. We are further justified in so dismissing the young of these hosts when we consider the three remaining which are the largest, namely, fox, brown hare and roe deer.

For the sake of argument let us (1) use maximum family-size figures; (2) ignore death-rate, and allow three litters per spring season in the case of hares; (3) assume the average individual cub, leveret or fawn has an infestation weight equal to the average lamb; in short, give the wild fauna the extreme maximum benefit of the doubt. Then (from last column, Table 5, assuming sex ratio unity except in sheep, the given population being four-fifths ewes) on the average hill farm we have to set about 150 young hares, twelve young foxes and three young roe deer against about 500 lambs. That is, young wild fauna would have a population host potential about one-third (165/500) that of the lamb population. Although adequate for our present purposes, this is, however, a fantastic overestimate. (1) Maximum family size is unusual. (2) Death-rate is higher the larger the family size; ecological pressure on the brown hare is so great that it is doubtful if more than one or two young in a litter survive for very long; and probably no more than two litters come into the picture during the spring tick season. (3) The greatest source of over-estimation lies in putting the others equal to the lamb in average individual infestation weight. At Lordenshaws, over the spring season of 1944, the average lamb maintained female ticks at 9 % of the figure for the average ewe (Milne, 1947*b*). This was for a mid-April peak of tick activity; with a May peak the lamb's percentage would be a little higher. The sheep equivalent of the average individual lamb is, therefore, of the order 0·10 during spring. This is not far short of the adult roe deer, greater than the adult hare and much greater than the adult fox (see Table 4). Indeed, on the reasonable assumption that the adult-young relation in sheep is not exceeded in the wild animals, then the average individual sheep equivalents would be roughly in the proportions, lamb : fawn : leveret : cub :: 50 : 7 : 4 : 1. With such proportions, and using mean family sizes, a little arithmetic reveals that the population host potentials ratio of all young wild hosts to lambs should be nearer 1/500 than 165/500 in the spring season in northern England. Hence, for our purposes, we can safely ignore young hosts in spring; indeed their inclusion would increase the comparative host potential of the sheep flock. And for north-east England, where little or no autumn activity obtains, this finding applies to the entire year.

In the autumn activity season of north-west England the surviving young of all eleven wild hosts to female ticks will take part. In effect the adult, or rather full-grown and nearly full-grown, population of wild hosts will have increased. But we doubt if this increase will be of very much moment. By autumn nature has usually taken heavy toll of the young; and, in the long run, populations of mature wild animals seem just to maintain themselves, in

the case of the larger mammals and birds at any rate. Moreover, before summer even, two-thirds to three-quarters of the annual female tick crop have already been fed. Hence the effect of wild host increase in autumn will be largely minimized on the background of the annual balance sheet. Even a trebled adult population of all wild host species in northern England would still leave the sheep flock supporting well over 90 % of the ground population of female ticks throughout the year (cp. Table 6).

For similar kinds of reasons we do not think that the single activity peak in summer, as in north-east Scotland (Moore, 1938, 1939), need upset our thesis where the eleven wild hosts are concerned.

Summing up: In confining our study to adult animals we do not seem to be underestimating the part played by wild hosts in maintaining the female tick population of northern England grazings, rather the reverse.

10. HOST POTENTIALS FOR NYMPHS AND LARVAE

The computations (Tables 7 and 8) in this section are less reliable than for female ticks (§ 9). For one thing, animal samples are more often inadequate. For another, population densities of some of the smaller animals are questionable. Perhaps the indirect calculation of some average individual sheep equivalents is also a weakness. On the whole, therefore, the present section should be regarded as a 'discussion', giving no more than a rough idea of the order of difference between wild fauna and sheep. For convenience, we focus upon the 'average' northern England farm shown in the last column of Table 5.

(i) *Adult hosts and nymphs*

At Hethpool during 1944, in weekly counts between 5 April and 13 June, the average nymphs to one female on sheep was about seven (6·99). In the same year, and at approximately the same times for Lordenshaws which has similar tick activity curves, we have weekly counts of female ticks on ten sheep and of all stages on ten rabbits (see Milne, 1947*a*, and Appendix 2 for details). On this farm, from 7 April to 23 June, the total of weekly means per sheep was 304·7 female ticks, and per rabbit 19·0 nymphal ticks. Hence one rabbit is equivalent to $19/304{\cdot}7 \times 1/6{\cdot}99 = 0{\cdot}0089$ of a sheep with respect to nymphs. Appendix 2 shows details of wild animal nymphal counts. For Hethpool, these compare direct with nymphs on sheep in 1944–6. For the other farms, whenever there are simultaneous rabbit counts, we can compute the sheep equivalent of a wild animal with respect to nymphs by taking one rabbit equal to 0·0089 of a sheep. Table 7 lists these sheep equivalents, for the average individual of some of the more important species, in their descending order (rank). As would be expected from § 6, the large majority of wild animals have a higher average individual sheep equivalent for nymphs than for female ticks. But there are five exceptions, namely, brown hare, roe deer, fox, stoat and badger. In the cases of brown hare and fox, at least, we cannot take this to be due to inadequate sampling; indeed we can suggest no explanation.

Table 7. *Sheep equivalent, with respect to nymphal ticks, of the average adult individual of certain wild species on hill farms*

(*Note.* Sample sizes differ from Table 3 in some cases owing to exigencies of calculations—see text.)

No. in sample	Species	Sheep equivalent of average individual
4	Hedgehog	0·4908
11	Pheasant	0·0835
18	Brown hare	0·0478
4	Roe deer	0·0451
16	Red grouse	0·0331
2	Weasel	0·0178
2	Partridge	0·0157
7	Fox	0·0156
4	Red squirrel	0·0141
16	Blackbird	0·0116
125	Rabbit	0·0089
1	Hedge-sparrow	0·0082
4	Stoat	0·0075
3	Long-eared owl	0·0074
2	Wheatear	0·0072
1	Redwing	0·0052
48	Skylark	0·0051
8	Magpie	0·0043
8	Lapwing	0·0026
65	Meadow-pipit	0·0016
23	Short-tailed vole	0·0013
6	Chaffinch	0·0009
10	Rook	0·0005
22	Carrion crow	0·0001

In addition, the following samples of wild animals yielded no nymphs when sheep were infested, usually at high or fairly high tick activity: 3 badgers, 5 brown rats, 16 long-tailed field mice, 2 bank vole, 42 common shrews, 16 common moles, 3 black grouse, 4 curlew, 10 wood-pigeons, 1 jay, 3 brown owls, 3 kestrels, 1 sparrowhawk, 3 woodcock, 10 snipe, 4 starlings, 5 thrushes, 1 redpoll, 1 coal-tit, 1 great tit, 2 robins, 2 whinchats, 3 pied wagtails, 4 willow-warblers, 1 swift. The otter samples (Table 3) was examined only for female ticks.

Table 8 gives population sheep equivalents with respect to nymphs for sixteen animals on the 'average' northern England hill farm of 1500 acres with 590 sheep. The populations of nine wild animals are as in Table 5. Those for weasel (20), common partridge (12) and long-eared owl (4) are derived from the same sources, i.e. gamekeepers, vermin

records, etc. Those for wheatear (45), skylark (233), lapwing (37) and meadow-pipit (635), are taken from Lack (1935) without adjusting for the small difference in acreage. Lack's figures are derived from sections of sixteen heath and moorland regions, totalling 1700 acres, eleven regions being in the southern half of England, one in Pembroke, two in Yorkshire, one in Ireland, and one in Shetland. His total acreage includes all the common moorland vegetation types. He states: 'No particular area of heathland in any region could be termed typical of that region. Nor between the wide limits already mentioned (3–224 adults per 100 acres) can any particular density of birds be considered typical for

Table 8. *Adult population sheep equivalents, with respect to nymphs, for sixteen wild animals on the 'average' hill sheep farm in northern England*

Species	Population sheep equivalent
Red grouse	10·66
Rabbit	6·16
Hedgehog	5·89
Pheasant	3·51
Skylark	1·19
Meadow-pipit	1·02
Brown hare	0·96
Weasel	0·36
Wheatear	0·32
Partridge	0·19
Roe deer	0·13
Stoat	0·12
Lapwing	0·10
Fox	0·06
Long-eared owl	0·03
Magpie	0·01
Total	30·71

British heathland.' *Faute de mieux*, however, we use Lack's totals for 1700 acres in order to form a rough idea. For the sixteen animals in Table 8 the total percentage of nymphs maintained in relation to sheep is $30{\cdot}7 \times 100/620{\cdot}7 = 4{\cdot}95\,\%$; and assuming the same order of error as in Table 6, the true percentage on the 0·05 probability, is probably a good deal less than 20 %, perhaps no greater than 10 % at the outside. But, although the sixteen animals include nearly all the important hosts, we are underestimating to some extent the contribution of the total wild fauna because of the following omissions.

The otter, since it is only an occasional visitor, cannot have a population sheep equivalent greater than fox or stoat, i.e. 0·1. Lack (1935) gives totals of 2 blackbirds, 16 hedge-sparrows, 2 chaffinches and 2 carrion crows on his 1700 acres, amounting to a total sheep equivalent of about 0·26. This might be a little higher, since Lack's figures are for areas devoid of bushes and trees, while the typical hill farm has 2–3 % of its area comprised of woodland and scrub and smaller aggregates of trees and bushes (§ 2), favouring higher populations of the first three birds. Rooks occasionally visit the hayfields and meadows of hill farms; but occasional visits cannot be of much consequence when 2000 individuals would require to live constantly on a farm to take the place of one sheep; moreover, occasional visiting would tend rather to deplete the tick population, the ticks picked up being dropped elsewhere. Seventy red squirrels equal one sheep; we cannot imagine that anything approaching this total could live in the small amount of woodland and scrub on the typical farm. Redwings leave the grazings some time before the height of the spring tick season, therefore their contribution will be small. In Table 7, twenty-five animals yielded no nymphs. All, except shrew and mole, if sampled in sufficient numbers, might reveal themselves hosts to nymphs in a small way. Lack also mentions about a score of smaller birds on his moors, some of which must be present in northern England, though not perhaps very common (we did not sample the small woodland species very thoroughly, see § 3). With one exception the above covers all the omitted animals claiming attention in the tick picture. Considering these few remarks, together with the trends appearing in Tables 7 and 8, their inclusion would undoubtedly necessitate raising our estimate of the wild fauna's contribution to nymph maintenance but, our strong impression is, not to any material extent in comparison to sheep.

There remains one host which may be important under certain conditions, namely, the short-tailed vole with its average individual sheep equivalent of 0·0013. This animal avoids bracken and gorse (Elton, 1942), and does not live in heather. It prefers rough grassland pasture. Thus, it is of no importance on heathery farms, nor on bracken areas of grassy farms. The following note on a conifer plantation (Elton, Davis & Findlay, 1935) in Border Country moorland is some help: 'Newcastleton State Forest is an almost isolated area of young conifer plantations set in surroundings of sheep pasture—mainly *Molinia* moor. The planted area in 1933 was about 2020 acres. Owing to the dense cover that grows up in absence of burning and sheep grazing, the forest carries a much higher vole population than the surrounding pastures. Various census observations indicate that during periods of abundance this planted area contains over a hundred thousand but less than half-a-million voles.' This maximum of 250 per acre confirms the gamekeeper's estimate of 200–300 roasted bodies per acre on Byrness State Forest after a fire. In certain types of country, voles seem to fluctuate between fairly wide levels every few years, building up and then 'crashing' to ten per acre or less. Even on hill grazings, there may occasionally be 'historic'

plagues, such as in the Border during 1875–6 and 1891–2, when to judge from Elton (1942) they may rise to several hundreds or perhaps even several thousands per acre on the sheep grazings. However, Elton (1942) says: 'Although most of the [British] work has been done inside the peculiar conditions of forest plantations, one has the strong impression that the [high] vole fluctuations are not often developed on open pasture. This we should expect from the obvious need of voles for food and cover and freedom from trampling and burning.' On the strength of these statements, we might put the average vole population of open rough-grass grazing ordinarily at somewhere between ten and fifty voles per acre,* the former figure being the approximate crash value and the latter the minimum abundance value in State Forest Plantations. Now grassy farms are more heavily stocked so that an average 1500-acre farm of this type would have 1000 rather than 590 sheep. Therefore the sheep equivalent of the vole population would be somewhere between 19·5 and 97·5, i.e. of nymphs supported by both voles and sheep, between 2 and 10 % would be supported by voles alone. Two considerations would, however, shorten the upper limit of this range considerably. First, most grassy farms now have large areas of bracken which voles avoid. Secondly, Elton (1942) states that it is 'probable that most populations of voles would be at their lowest seasonal ebb in spring and their highest point in the autumn'; while the major part of nymphal activity in northern England takes place in spring and early summer.

Summing up we may say that while the adult wild fauna support, proportionately, considerably more nymphs than they do females in comparison with adult sheep, nevertheless, the sheep are by far the major hosts for nymphs. Even where voles are likely to be numerous, on suitable areas of grassy farms, this is still likely to be true.

(ii) *Adult hosts and larvae*

Unfortunately, we have no detailed larval counts for sheep. Nor, since the nymphs : larvae ratio varies with size of host (see Table 2), can we safely compute such a ratio for sheep on the basis of results for smaller mammals. The trend of observations on wild animals (cp. Table 1) suggests forcibly, however, that the sheep must outstrip enormously all northern England wild hosts in average individual larval infestation weight. Certainly that was our strong impression gained while handling sheep for counts of other stages. Remembering the relation between host size and tick stage infesting, as well as other points in §§ 4, 6, 8 and in (i) above, we can also see that while sheep have more rival (wild) hosts for nymphs than for females, the rival host pressure is even greater for larvae. For example, we have only to think of the many small creatures, including birds and mice and multitudes of shrews, on a farm which do not, or barely, come into the nymph picture. Hence, while we do not suggest that the adult wild fauna comes near rivalling the adult sheep flock in maintaining larvae, we can safely advance the idea that the population sheep equivalent of the adult wild fauna increases from female to nymph and from nymph to larva.

(iii) *Young hosts included*

In the adult wild animals we have just seen that population sheep equivalent increases from female to nymph and from nymph to larva. Obviously, the same applies in the case of the populations of their young. But the gradient of increase will be steeper since the smaller the host, generally the larger the family and the more families per season. Bearing death-rate considerations in mind, however, we think it reasonable to conclude that when young are included: (1) The sheep flock has considerably the major host potential for nymphs, although the proportion supported by the wild fauna will be rather higher than suggested in para. (i) above. (2) In supporting larvae the wild fauna, though more important than in the case of nymphs, will still have the minor host potential.

We should point out that, although unimportant as hosts to female ticks, the wild fauna by feeding nymphs and larvae in any degree is part-provider of the females which infest sheep. In this respect, however, its part (1 and 2 above) is of course minor compared with that of the sheep themselves.

11. GENERAL DISCUSSION AND CONCLUSIONS

In such a diffuse subject there are bound to be some controversial issues; but there can be no doubt that the part played by the wild fauna in supporting (i.e. feeding) female ticks is very small, indeed negligible, in comparison to the sheep flock on the five farms studied. Since these farms are representative, and since there are no other hosts (except perhaps a few red deer somewhere in Lakeland), this is probably true of northern England as a whole. In any case, if animal populations are known, the problem could be roughly worked out for any farm by using the estimated average individual sheep equivalents in Table 4. We must consider, however, how far the latter are generally applicable. Do they vary with faunal density composition? For instance, if a wild species is twice as numerous as sheep on one farm and only half on another, will the average individual sheep equivalent of that species be the same on both farms? This is a complex question. Unfortunately our data (Appendix 2) are not

* See footnote to p. 184, col. 1.

sufficiently comprehensive to permit a direct, factual answer, but we discuss the problem below.

Each animal on a grazing takes its share out of the common pool of ticks on the ground. This share will vary directly according to the area of ground it covers (sweeps) in unit time. Let us imagine two herbivorous species. *A* (the larger) and *B*, both living on the same food plants, but, on the average, the individual of *A* requiring say five times as much food as the individual of *B*. Now, a pasture, or rather, the palatable parts of it, receives uniform attention throughout from grazers. Hence, no matter what the relative sizes of their populations, individuals of *A* and *B* on the same pasture will always have to cover areas in the ratio 5 : 1 in order to collect sufficient food, i.e. their infestation levels will always be in the ratio 5 : 1. Apart from sampling error then, the average individual sheep equivalent of any herbivorous species should be the same whatever the faunal density composition. But the question is more complex in the case of carnivores. Obviously, the peregrinations of a fox and a sheep are not influenced directly by the same food factor. Accordingly there may be some variation in average individual sheep equivalent for carnivores according to faunal density composition. We cannot state what this variation would amount to, but judging from our limited experience on the five farms it should not be great. No doubt we have over-simplified the issues. Nevertheless, in order materially to change our picture of the relative parts played by total wild fauna and sheep flock, the average individual sheep equivalents (Table 4) would require to be unreasonably greater than we have estimated. On the other hand, we think that such equivalents for hedgehog (Tables 4 and 7) have been overestimated. A hedgehog taken from its nest at Hethpool in September had a very high infestation, indeed as high as that of another taken during peak spring activity of the same year (Milne, 1947*a*, and Appendix 2). Now tick activity on the pastures of this farm is invariably very low or zero in September, as shown by sheep and blanket counts over several years. The only possible explanation, therefore, is that, as with *Ixodes hexagonus* Leach (Elton, 1937), the hedgehog carries on a traffic in *I. ricinus* within its nest. In that case a hedgehog tick count comprises not only ticks picked up from the general pasture but also those from its nest. Accordingly its sheep equivalent, calculated from a total count, will be too high because the sheep has only the former source of infestation.

In dealing with host potentials, except for rook (§ 10), we ignored the question: how far do some animals abstract ticks from a grazing? For instance, animals like roe deer and certain birds must drop some of their ticks in the neighbouring woods barred to sheep. Obviously, the contributions of such animals to the maintenance of the tick population of the general sheep grazing could then only be less, not more, than their actual infestations relative to sheep would indicate. This merely fortifies our thesis that sheep support the vast majority of ticks on a grazing.

From our findings on female ticks in northern England, what are the implications for British hill and moorland grazings in general? This depends on how wild animal populations vary throughout Britain and on whether there are additional female tick hosts in other parts of the country. Obviously, no firm answer can be given in the absence of detailed information on these two points. But the following is our opinion arising from discussions with gamekeepers and our own knowledge of fairly representative areas in different parts of Scotland, Wales and England.

For the eleven female tick hosts studied in northern England, probably the highest densities among our five farms are normally not greatly exceeded on tick-infested hill-sheep grazings anywhere in Britain except in the cases of brown hare and perhaps hedgehog.

Other things being equal, brown hares would reach their maximum on the best moors, i.e. where sheep run at one per acre. Even if they attained the utter maximum of one hare to five acres, 'permitted on a well-managed moor' (see Part 1), this would only give a population equivalent of 3 % of the sheep flock at the outside, with respect to female ticks. As our figures attest, however, brown hares are never numerous on the general run of hill and moorland grazings, even in the absence of intensive human persecution. Unlike Scottish mountain hares, they prefer richer pastures at a lower level. Accordingly, we think that brown hares are seldom, if ever, an important factor in the maintenance of female ticks on hill-sheep grazings.

Shcheglova (1939) puts the hedgehog at the head of his wild hosts, the biggest of which was the polecat (deer, hares, rabbits, foxes, stoats, badgers, etc. being apparently absent on his grazings). He goes so far as to say: 'We may take it that the main body of ticks parasitizing wild vertebrate animals occurs in hedgehogs.' Even in the absence of larger wild hosts, this could only be true, of course, if he found much higher populations of hedgehogs than we did; he does not give population figures, but the description of his 'forest pastures' as largely open woodland and scrub does suggest very favourable hedgehog country. Against all this we must place the facts that its average individual sheep equivalent may be fairly grossly overestimated, it misses the main part of the spring tick season, and on most hill grazings in Britain it is scarce because the habitat is unfavourable. In short, we are inclined to rate the hedgehog

lower than Shcheglova. Nevertheless, it is important and deserves further study.

As to additional hosts in other parts of Britain, we must consider eight animals in the light of our finding that female ticks are confined to the larger mammals and larger birds in the wild fauna. Two of these, red deer and Scottish mountain hare, are known hosts and have already been discussed in detail (Part 1) on the basis of the limited data available. Both animals require further investigation, but meanwhile the following additional comments may not be out of place.

Scottish mountain hare, as the name implies, is practically confined to Scotland and there on the highest grazings only. It has been introduced to at least one moor south of the border (north Wales, according to a private communication from Mr C. Elton). We doubt if its average individual sheep equivalent differs much from that for brown hare, despite Moore's general statement (Part 1). Abnormally high populations of mountain hare, however, occur more frequently, probably because of the inaccessibility of their haunts and the larger areas under the supervision of gamekeepers. For instance (perhaps extreme), Moore cites a grazing where mountain hares were about twice as numerous as sheep. In such a case, if their average individual sheep equivalent were no greater than that for brown hares, they would be supporting female ticks to the extent of something between 7 and 26 % of the sheep figure.

The red deer, important in Scotland, is said to occur also on the moors of Devon and Cornwall. Seemingly, a red deer feeds more female ticks than a sheep; and the normal red deer population of an area may have a host potential of the same order as the normal sheep population for a similar area (Part 1).

There remain only five of the larger British wild mammals not investigated, namely, pine marten or *Martes martes*, polecat or *Mustela putorius*, wild cat or *Felis silvestris*, fallow deer or *Dama dama*, and grey squirrel or *Sciurus carolinensis*. Judging from our findings on populations of fox, badger, otter and stoat, we can safely rate the parts played by marten, polecat and wild cat as negligible singly and collectively, in view of their general scarcity. Again, our findings for red squirrel do not suggest that grey squirrel need be considered seriously, and fallow deer, like roe deer, need not be reckoned with on typical hill and moorland grazings.

The capercaillie, or *Tetrao urogallus*, is the only 'larger' bird requiring consideration. Moore (see Part 1) records this bird as host to immature ticks. Probably its relation to female ticks is at best only similar to that of other game birds, i.e. unlikely to increase the part played by total wild hosts materially in comparison with the sheep flock.

On the applied side, i.e. on the question of tick control on hill and moorland grazings, it seems, therefore, that we may conclude as follows:

(1) The tick will parasitize any mammal or bird it may meet. Under existing wild and domestic faunal conditions in Britain, this catholicity ensures not only its survival as a species but also its obvious success in building up and maintaining efficient populations in places where the ground microhabitat is suitable.

(2) In control attempts the most efficient attack can be made on the female stage of the tick (§ 3). There is no evidence that any of the wild hosts to this stage in northern England, bird or mammal, merits extirpation (as Moore, see Part 1) or even reduction in numbers as an adjunct to other control measures. Over Britain, as a whole, it may be that mountain hares and red deer, alone among all the wild hosts, might profitably be thinned under certain conditions in a few districts.

(3) Given that acaricidal treatment could be applied to sheep (see Milne, 1945*c*) so that no attaching female ticks escaped, then, on grazings lacking red deer and/or abnormal populations of mountain hare, the tick life cycle could be cut to the extent of 94–99 % of the breeding females annually, whence control could not fail to follow. Withdrawal of sheep would not have so quick an effect. The immediate result of the latter would be a greatly increased infestation level for all the wild female tick hosts (cf. Milne, 1945*b*) and the limit to host size for female ticks might be temporarily lowered. In due course, infestation level would fall but, unless kept down, wild hosts would increase in the absence of sheep. In a very much reduced population, male and female ticks might have some difficulty in meeting for mating purposes. Teesdale (1940) did not find this in the case of the tsetse fly, *Glossina palpalis*; but the tick does not fly and the two sexes can only meet on hosts; in a meagre tick population, a male tick might get on a host which failed to pick up a female, and vice versa. Nevertheless, for the reason given in (1) above, it is unlikely that the tick could be completely eradicated on any grazing, even by the most efficient and persistent practicable treatment of a resident sheep flock. But its populations could be reduced to comparatively very low levels.

12. SUMMARY

Although three other *Ixodes* species were found, *Ixodes ricinus* L., when it occurs, is by far the most important tick parasite of wild and domestic animals on hill and moorland grazings in northern England. In the following, 'tick' or 'ticks' refer to *I. ricinus*.

The previous list of British mammal hosts, wild and domestic, to the tick (any stage) is largely

confirmed, and increased approximately from twenty-three to twenty-nine species; and birds, similarly, from twenty-one to thirty-nine species. The preponderance of small mammals and birds among the twenty-four new-recorded host species confirms the impression that previous workers tended to confine their attentions to the larger animals. The host list is still incomplete, and the suggestion that the tick will parasitize any bird or mammal it may meet is strongly reinforced.

No host occurs below the reptile level among vertebrates; and among British reptiles, only the common lizard is a host—rarely. There appear to be no hosts among invertebrates.

All the farm and domestic mammals from horse down to cat are hosts to female ticks, but poultry have never been recorded—probably because they do not much frequent the tick-infested pastures.

Only the larger wild mammals and birds are hosts to female ticks. In northern England these include eight mammals and three birds. For Britain as a whole two other mammals are known, while a further four mammals and one bird are likely hosts.

With or without the inclusion of domestic animals, proportionately more mammal than bird species are hosts to female ticks. This is because there are more mammal than bird species large enough to be hosts.

All the recorded British hosts, except shrew and possibly mole, are hosts in some degree to nymphs, and all, without exception, are hosts to larvae.

A certain amount of self-deticking is practised by birds, and very probably by some of the smaller mammals. Birds never have ticks attached within beak-reach.

Expectedly, larvae are seasonally more numerous than nymphs on hosts, and nymphs more numerous than females.

The smaller the host, the fewer the female ticks relative to nymphs, and the fewer the nymphs relative to larvae. The reason was not discovered.

In any host species there is high variation in infestation weight among individuals going on the same ground at the same time.

In general, the smaller the host the lower the average infestation weight of female ticks; the same is true of both nymphs and larvae. This is most likely to be a function of the amount of ground covered in unit time; the smaller the host, generally the less ground covered and hence the fewer ticks met. In detail, however, there are plenty of exceptions since habits are diverse.

The sheep is the chief farm-animal host on hill grazings. The individual sheep feeds very many more female ticks than the most heavily infested wild host (red deer excepted).

On northern England hill grazings the percentage of the female tick ground population fed by all the wild hosts lies somewhere between 0·8 and 5·6 %. That is, the sheep flock feeds 94–99 % of the female ticks, the higher figure being more probably nearer the truth.

Proportionately, the wild hosts support more of the nymphal and still more of the larval ground population than they do of the female. Wild hosts are, therefore, to some not altogether inconsiderable extent, part-providers of the female ticks which infest sheep. The sheep flock, however, is very probably still the major supporter of the larval population, and certainly considerably the major supporter of the nymphal.

Attempts at tick control are, rightly, concentrated upon the female stage of the tick. When the above findings are applied to the problem the following points emerge:

In northern England (i) birds are of no importance as female hosts; (ii) nor is there any evidence of necessity to extirpate or even reduce any of the wild mammal host populations; (iii) if acaricidal treatment were serially applied to hill flocks so that no attaching females escaped, the breeding section of the tick ground population would be reduced by over 94 % per annum, whence control could not fail to follow; (iv) the tick has such a wide range of hosts that its complete eradication could not be expected.

Over Britain, as a whole, it seems very probable that these four points still hold good with one proviso, namely, that further study may point the necessity for thinning out the red deer and mountain hare populations in some few areas of Scotland.

This work could not have been carried out without the great goodwill and actual physical help of many people in laboratory and field. Warm thanks are due to: (1) His Grace the Duke of Northumberland, Captain the Hon. Claud Lambton, J. R. Blackett-Ord, Esq., F. J. Moralee, Esq., B. Armstrong, Esq., and the Forestry Commission, among other landowners and farmers in northern England, who gave the writer every needed facility on and with their property and showed lively interest in the findings; (2) several gamekeepers including J. Rogerson, Esq., A. Chave, Esq., and J. Hutchison, Esq., who went to no end of trouble to secure the wild-animal specimens and provide population figures; (3) several shepherds including T. Dunn, Esq., E. Douglass (deceased), W. Murray, Esq., and B. Armstrong (Jnr.) who for years gathered their sheep weekly, frequently at great inconvenience, for tick counts, and helped in collecting wild animal specimens; (4) the Northumberland County War Agricultural Executive Committee, particularly A. Dawson, Esq., Pest Officer, who seconded J. Rogerson to work on Lordenshaws and kept us supplied with cartridges when the latter were almost unobtainable; (5) J. Allan Campbell, Esq., M.R.C.V.S., B.Sc., for data on reptiles;

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APPENDIX 1

List of wild animals examined during tick activity

The list shows total specimens, common names and specific names. Nomenclature follows the *British Museum List of British Vertebrates* (1935). The figures in parentheses show total infested specimens:

17 common moles (2)	*Talpa europaea*
42 common shrews (28)	*Sorex araneus*
5 hedgehogs (5)	*Erinaceus europaeus*
25 foxes (9)	*Vulpes vulpes*
31 fox cubs (2)	*V. vulpes*
9 badgers (1)	*Meles meles*
2 otters (1)	*Lutra lutra*
5 stoats (2)	*Mustela erminea*
2 weasels (0)	*M. nivalis*
317 rabbits (162)	*Oryctolagus cuniculus*
473 rabbits examined for ♀♀ ticks only	*O. cuniculus*
21 brown hares (17)	*Lepus europaeus*
2 bank voles (0)	*Clethrionomys glareolus*
23 short-tailed voles (10)	*Microtus agrestis*
17 long-tailed field mice (11)	*Apodemus sylvaticus*
5 brown rats (0)	*Rattus norvegicus*
4 red squirrels (4)	*Sciurus vulgaris*
8 roe deer (8)	*Capreolus capreolus*
45 carrion crows (1)	*Corvus corone*
11 rooks (2)	*C. frugilegus*
11 magpies (6)	*Pica pica*
1 British jay (0)	*Garrulus glandarius*
4 starlings (1)	*Sturnus vulgaris*
6 chaffinches (2)	*Fringilla coelebs*
1 lesser redpoll (0)	*Acanthis flammea*
48 skylarks (35)	*Alauda arvensis*
3 pied wagtails (0)	*Motacilla alba*
67 meadow-pipits (40)	*Anthus pratensis*
1 British great titmouse (1)	*Parus major*
1 British coal-titmouse (0)	*P. ater*
4 willow-warblers (1)	*Phylloscopus trochilus*
5 British song-thrushes (1)	*Turdus ericetorum*
1 redwing (1)	*T. musicus*
16 blackbirds (7)	*T. merula*
2 British robins (1)	*Erithacus rubecula*
2 whinchats (2)	*Saxicola rubetra*
2 wheatears (1)	*Oenanthe oenanthe*
1 British hedge-sparrow (1)	*Prunella modularis*
1 swift (0)	*Micropus apus*
3 long-eared owls (2)	*Asio otus*
3 tawny owls (0)	*Strix aluco*
1 sparrow-hawk (0)	*Accipiter nisus*
4 kestrels (1)	*Falco tinnunculus*
3 woodcock (0)	*Scolopax rusticola*
10 common snipe (4)	*Capella gallinago*
5 curlew (0)	*Numenius arquata*
8 lapwings (2)	*Vanellus vanellus*
12 wood-pigeons (0)	*Columba palumbus*
11 pheasants (9)	*Phasianus colchicus*
3 common partridges (2)	*Perdix perdix*
3 British black grouse (1)	*Lyrurus tetrix*
21 British red grouse (13)	*Lagopus scoticus*
4 common lizards (0)	*Lacerta vivipara*
14 adders (0)	*Vipera berus*
4 common frogs (0)	*Rana temporaria*
3 common toads (0)	*Bufo bufo*

APPENDIX 2

Details of observations in northern England during 1943–6

Showing location, date of collecting, sample size, animal species and tick yield (*Ixodes ricinus* L.). ♂♂, males; ♀♀, females; o, nymph; oo, nymphs; L, larva; LL, larvae. Tick yield is given as total from all the individuals in the sample. All the tick stages were counted in the case of the wild fauna. Except at Hethpool, only female ticks were counted on sheep. At Hethpool, both females and nymphs were counted on sheep in 1944–6. The sheep counts are for ewes, usually in actual fact but sometimes transformed hogg (1-year-old sheep) counts (see Milne, 1947*b* for transformation factors). Apart from fox (some cubs being included as shown), rabbit (a small proportion were three-quarters grown—not indicated) and 'wild' goat (two kids included as shown), all the wild animals were adult—at least in size.

(i) *Lordenshaws*

1943

9 *April*. 10 sheep: 752 ♀♀; 1 weasel: nil; 20 rabbits: 3 ♂♂, 2 ♀♀, 8 oo, 1 L; 1 carrion crow: nil.

16 *April*. 10 sheep: 1069 ♀♀; 4 common moles: nil; 15 rabbits: 3 ♀♀, 11 oo, 2 LL; 2 carrion crows: nil; 4 rooks: nil; 1 magpie: 1 ♀, 4 oo, 1 L; 1 wheatear: nil.

23 *April*. 10 sheep: 690 ♀♀; 1 fox: 2 ♀♀; 1 badger: 3 ♂♂, 13 ♀♀, 2 oo; 3 rabbits: 5 ♀♀; 4 carrion crows: nil; 1 magpie: 4 oo; 1 kestrel: nil; 1 red grouse: nil.

30 *April*. 10 sheep: 486 ♀♀; 2 common moles: nil; 1 badger: nil; 3 rabbits: 37 oo; 1 carrion crow: nil; 3 rooks: nil; 2 magpies: 1 o; 1 song-thrush: nil; 2 wood-pigeons: nil; 1 pheasant (sick): 4 ♀♀, 38 oo.

7 *May*. 10 sheep: 470 ♀♀; 1 stoat: 2 ♂♂, 8 ♀♀, 1 o.

14 *May*. 10 sheep: 211 ♀♀; 1 common shrew: nil; 5 carrion crows: nil; 2 magpies: nil.

21 *May*. 10 sheep: 160 ♀♀; 1 common shrew: nil; 1 fox: nil; 3 fox cubs: nil; 1 stoat: nil; 10 rabbits: 17 oo, 7 LL; 9 short-tailed voles: 5 LL; 7 carrion crows: 2 oo, 3 LL; 1 magpie: 2 oo, 1 L; 1 kestrel: nil; 1 curlew: nil.

28 *May*. 10 sheep: 102 ♀♀; 1 fox cub: 2 ♂♂, 2 ♀♀; 2 rabbits: nil; 3 carrion crows: nil; 1 rook: nil.

16 *September*. 10 sheep: 6 ♀♀; 2 rabbits: nil; 1 brown hare: 1 o; 2 meadow-pipits: nil; 4 red grouse: 1 o; 1 adder: nil.

1944

31 *March*. 10 sheep: 313 ♀♀; 7 rabbits: 2 oo; 1 skylark: nil; 1 woodcock: nil.

7 *April*. 10 sheep: 148 ♀♀; 3 common moles: nil; 10 rabbits: nil; 1 long-tailed field mouse: nil; 2 skylarks: nil; 1 meadow-pipit: nil; 1 robin: nil; 2 lapwings: nil.

14 *April*. 9 sheep: 599 ♀♀; 10 rabbits: 1 ♀, 26 oo, 3 LL; 1 long-tailed field mouse: 2 LL.

21 *April*. 9 sheep: 763 ♀♀; 1 common shrew: nil; 10 rabbits: 17 oo, 19 LL; 2 brown hares: 5 ♂♂, 5 ♀♀, 1 L; 1 short-tailed vole: 2 LL; 1 long-tailed field mouse: nil; 5 carrion crows: nil; 1 magpie: 2 oo; 1 chaffinch: 3 LL; 3 skylarks: 1 L; 7 meadow-pipits: 1 o; 1 redwing: 1 o; 2 tawny owls: nil; 2 common snipe: nil; 1 lapwing: nil; 1 pheasant: 1 ♀, 2 oo, 1 L; 2 partridges: 6 oo; 1 black grouse: nil; 1 red grouse: 1 ♀, 4 oo, 3 LL.

28 *April*. 9 sheep: 324 ♀♀; 2 common moles: nil; 7 common shrews: 23 LL; 1 stoat: nil; 12 rabbits: 1 ♀, 35 oo, 17 LL; 1 short-tailed vole: 29 LL; 7 long-tailed field mice: 31 LL; 1 red squirrel: 1 o; 4 carrion crows: nil; 1 rook: nil; 1 magpie: 1 o; 1 chaffinch: nil; 1 skylark: 1 o; 1 pied wagtail: nil; 4 meadow-pipits: nil; 1 coal-tit: nil; 1 tawny owl: nil; 2 common snipe: nil; 1 curlew: nil; 1 lapwing: nil; 2 wood-pigeons: nil; 4 pheasants: 2 ♀♀, 61 oo, 20 LL; 1 black grouse: 1 L; 3 red grouse: 15 oo.

5 *May*. 8 sheep: 168 ♀♀; 2 common shrews: 1 L; 10 rabbits: 1 ♀, 47 oo, 74 LL; 1 red squirrel: 1 o; 2 carrion crows: nil; 1 skylark: 4 LL; 4 meadow-pipits: 1 o, 10 LL; 1 song-thrush: 8 LL; 2 common snipe: nil; 1 curlew: nil; 1 lapwing: nil.

12 *May*. 9 sheep: 239 ♀♀; 13 rabbits: 14 oo, 94 LL; 2 short-tailed voles: 4 LL; 2 carrion crows: nil; 1 rook: 1 o; 1 jay: nil; 1 chaffinch: nil; 1 skylark: 3 oo, 7 LL; 3 meadow-pipits: 1 o, 10 LL; 1 hedge-sparrow: 1 o, 1 L; 1 kestrel: 1 L; 1 curlew: nil; 1 lapwing: nil; 1 pheasant: nil (sitting close on eggs); 2 adders: nil.

19 *May*. 9 sheep: 179 ♀♀; 4 common shrews: 5 LL; 1 fox cub: nil; 10 rabbits: 24 oo, 120 LL; 4 short-tailed voles: nil; 3 long-tailed field mice: nil; 2 chaffinches: nil; 1 lesser redpoll: nil; 1 skylark: 1 L; 2 pied wagtails: nil; 5 meadow-pipits: 2 LL; 2 willow-warblers: 1 L; 2 whinchats: 3 LL; 1 sparrow-hawk: nil; 1 woodcock: nil; 1 red grouse: 1 L.

26 *May*. 9 sheep: 149 ♀♀; 3 fox cubs: nil; 10 rabbits: 19 oo, 189 LL; 1 short-tailed vole: nil; 1 long-tailed field mouse: 4 LL; 1 rook: 3 LL; 1 chaffinch: 1 o, 2 LL; 1 skylark: 23 LL; 3 meadow-pipits: 1 o; 18 LL; 1 song-thrush: nil; 2 blackbirds: 1 o, 9 LL; 2 snipe: 8 LL; 1 wood-pigeon: nil.

2 *June*. 9 sheep: 82 ♀♀; 1 common shrew: 3 LL; 1 fox: nil; 10 rabbits: 5 oo, 94 LL; 1 long-tailed field mouse: 5 LL; 1 brown rat: nil; 1 red squirrel: 4 oo, 18 LL; 2 carrion crows: nil; 1 magpie: nil; 4 skylarks: 5 oo, 61 LL; 5 meadow-pipits: 1 o, 59 LL; 1 great tit: 4 LL; 1 blackbird: 3 oo, 3 LL; 1 robin: 2 LL; 1 wheatear: 1 o, 2 LL; 1 kestrel: nil; 1 common snipe: 2 LL; 1 lapwing: 2 oo; 1 pheasant: 15 oo, 36 LL.

9 *June*. 9 sheep: 43 ♀♀; 4 common shrews: 1 L; 10 rabbits: 28 LL; 1 brown rat: nil; 1 red squirrel: 7 oo, 26 LL; 2 skylarks: 19 LL; 3 meadow-pipits: 10 LL; 1 long-eared owl: 1 o, 1 L.

16 *June*. 9 sheep: 34 ♀♀; 1 common mole: 2 LL; 2 common shrews: nil; 2 badgers: nil; 10 rabbits: 11 oo, 101 LL; 1 long-tailed field mouse: 2 LL; 4 starlings: 2 LL; 4 skylarks: 1 o, 57 LL; 5 meadow-pipits: 2 oo, 50 LL; 1 blackbird: 1 o, 9 LL; 2 long-eared owls: 1 L; 3 wood-pigeons: nil; 1 red grouse: 2 oo, 3 LL.

23 *June*. 10 sheep: 9 ♀♀; 1 common mole: 2 LL; 1 common shrew: 6 LL; 1 weasel: nil; 1 short-tailed vole: 1 L; 10 rabbits: 1 o, 76 LL; 2 brown rats: nil; 4 skylarks: 1 o, 40 LL; 6 meadow-pipits: 65 LL; 1 song-thrush: nil; 1 swift: nil.

30 *June*. 9 sheep: 16 ♀♀; 10 rabbits: 2 oo, 28 LL; 1 skylark: 1 o, 3 LL; 3 meadow-pipits: 19 LL; 7 blackbirds: 1 L.

7 *July*. 9 sheep: 19 ♀♀; 4 rabbits: 10 LL.

21 *July*. 8 sheep: 19 ♀♀; 10 rabbits: 10 LL; 3 skylarks: 2 oo, 10 LL; 3 blackbirds: 1 o, 1 L.

4 *August*. 9 sheep: 7 ♀♀; 3 meadow-pipits: 10 LL; 2 blackbirds: nil.

18 *August*. 9 sheep (females): nil; 10 rabbits: 3 oo, 8 LL; 4 skylarks: 2 LL; 1 wood-pigeon: nil.

1 *September*. 9 sheep: 2 ♀♀; 10 rabbits: 6 LL; 3 skylarks: nil.

15 *September*. 8 sheep: 3 ♀♀; 10 rabbits: 12 oo, 10 LL; 2 meadow-pipits: 1 L; 2 red grouse: 1 o.

29 *September*. 9 sheep: 8 ♀♀; 10 rabbits: 1 o, 4 LL.

13 *October*. 7 sheep: 6 ♀♀; 1 stoat: nil; 10 rabbits: 3 oo, 1 LL; 2 skylarks: nil; 3 meadow-pipits: nil; 1 song-thrush: nil; 1 woodcock: nil; 1 red grouse: nil.

20 *October*. 1 fox: nil; 10 rabbits: 1 o; 1 brown hare: nil; 2 meadow-pipits: nil.

27 *October*. 9 sheep: 1 ♀.

3 *November*. 10 rabbits: 2 oo, 1 L; 2 meadow-pipits: nil; 1 wood-pigeon: nil; 1 black grouse: nil; 1 red grouse: nil.

1945

28 *March*. 10 sheep: 259 ♀♀.

6 *April*. 10 sheep: 201 ♀♀.

13 *April*. 10 sheep: 309 ♀♀; 1 pheasant: 35 oo, 16 LL.

27 *April*. 10 sheep: 192 ♀♀; 1 brown rat: nil; 3 skylarks: 2 oo, 2 LL; 1 meadow-pipit: 1 o, 1 L; 2 willow-warblers: nil; 1 pheasant: 78 oo, 2 LL; 1 red grouse: 33 oo, 4 LL.

4 *May*. 10 sheep: 88 ♀♀; 2 foxes: nil; 2 carrion crows: nil.

11 *May*. 10 sheep: 138 ♀♀; 1 common mole: nil; 1 fox: nil; 1 carrion crow: nil.

18 *May*. 10 sheep: 77 ♀♀.

25 *May*. 10 sheep: 76 ♀♀; 2 common moles: nil; 1 magpie: nil; 3 skylarks: 7 oo, 6 LL; 2 meadow-pipits: 2 oo, 2 LL; 1 pheasant: 1 ♀, 16 oo, 11 LL.

1 *June*. 10 sheep: 23 ♀♀; 1 stoat: 4 oo, 2 LL; 2 skylarks: 8 oo, 32 LL; 1 red grouse: 4 oo.

22 *June*. 10 sheep: 2 ♀♀; 2 skylarks: 1 o, 12 LL; 1 meadow-pipit: 5 LL; 1 common snipe: 1 L; 2 red grouse: 3 LL.

Note. Numerous ground beetles were caught in the vole traps (tin canisters). No ticks were found on beetles.

1946

19 *April*. 10 sheep: 754 ♀♀; 2 brown hares: 2 ♂♂, 13 ♀♀, 5 oo, 1 L.

(ii) *Hethpool*

1943

7 *April*. 10 sheep: 612 ♀♀; 20 rabbits: nil.

1944

5 *April*. 10 sheep: 44 ♀♀, 72 oo; 10 rabbits: 14 oo.

13–18 *April*. 9 sheep: 102 ♀♀, 397 oo; 3 common shrews: 17 LL; 1 fox: 1 ♂, 2 ♀♀; 1 bank vole: nil.

20–24 *April*. 10 sheep: 127 ♀♀, 430 oo; 1 'wild' goat kid: 1 ♂, 10 ♀♀, 7 oo, 2 LL; 8 common shrews: 34 LL; 1 bank vole: nil.

28 *April*–1 *May*. 9 sheep: 198 ♀♀, 1094 oo; 4 common shrews: 42 LL; 1 short-tailed vole: 1 o, 8 LL.

5–8 *May*. 9 sheep: 162 ♀♀, 980 oo; 1 'wild' goat kid: 7 ♂♂, 15 ♀♀, 7 oo, 1 L; 1 brown hare: 1 o, 11 LL.

14–15 *May*. 10 sheep: 125 ♀♀, 885 oo; 1 rabbit: 1 o, 6 LL.

30 *May*–3 *June*. 9 sheep: 105 ♀♀, 935 oo; 1 common shrew: 3 LL; 2 rabbits: 2 oo, 46 LL; 3 short-tailed voles: 136 LL.

8–15 *June*. 10 sheep: 82 ♀♀, 1148 oo; 2 common shrews: 19 LL; 1 hedgehog: 1 ♀, 13 oo, 90 LL.

11 *September*. 1 hedgehog: 13 oo, 88 LL (found under hay cock—warmth causing tick activity? No female ticks on sheep and very few nymphs).

1945

9–10 *April*. 9 sheep: 866 ♀♀, 3205 oo; 1 fox: 1 ♂, 4 ♀♀, 1 o.

12–14 *May*. 10 sheep: 169 ♀♀, 929 oo; 1 adder: nil.

26–28 *May*. 9 sheep: 89 ♀♀, 476 oo; 1 hedgehog: 2 ♂♂, 3 ♀♀, 52 oo, 999 LL.

9–10 *June*. 9 sheep: 40 ♀♀, 128 oo; 1 hedgehog: 57 oo, 55 LL (this hedgehog escaped from its tick-collecting cage on the first night; hence probably a few ticks lost).

1946

28–31 *March*. 10 sheep: 469 ♀♀, 155 oo; 1 brown hare: 10 ♂♂, 11 ♀♀, 15 oo, 3 LL.

21–23 *May*. 10 sheep: 315 ♀♀, 456 oo; 1 brown hare: 2 ♂♂, 4 ♀♀.

(iii) *Byrness Forestry Area*

This area has a tick activity season similar to Lordenshaws and an infestation level about the same. Except in the case of reptiles and amphibians (done by the writer; other animals by the gamekeeper) no thorough search was made for nymphs and larvae. The data for adult ticks, at least females, are, however, dependable.

1943

1 *April*–20 *May*. 8 foxes, 9 fox cubs, 1 long-tailed field mouse, 4 carrion crows, 1 curlew and many rabbits: all with no female ticks.

5 *April*. 1 brown hare: 4 ♂♂, 13 ♀♀.

12–15 *April*. 1 fox: 2 ♂♂, 4 ♀♀; 1 otter: no female ticks; 1 roe deer: 6 ♂♂, 11 ♀♀.

18–19 *May*. 1 'wild' goat and 2 wood-pigeons: all with no female ticks.

August. 2 badgers, 1 in first week, 1 in third week: both with no female ticks.

1944

3 *May*. 1 otter: 3 ♂♂, 7 ♀♀.
27 *April*–26 *May*. 473 rabbits: no female ticks.
9 *May*–23 *May*. 2 foxes: a few female ticks.
24 *May*–13 *June*. 2 foxes: a few female ticks; 9 fox cubs: no female ticks.
13–27 *June*. 3 foxes: one or two female ticks on one fox only; 4 fox cubs: no female ticks.
26 *June*. 1 badger: no female ticks.
5 *August*. 1 badger: no female ticks.
15 *August*. 1 common partridge: no female ticks.

1945

10 *May*. 2 common toads: nil.
5 *June*. 3 common frogs: nil.
15 *June*. 1 hedgehog: 1 ♀.
20 *June*. 1 adder: nil; 1 common toad: nil.

1946

5 *April*. 1 roe deer: 1 ♂, 3 ♀♀.
28 *April*. 1 roe deer: 2 ♀♀.
8 *May*. 1 brown hare: 1 ♂, 4 ♀♀, 8 oo.
11–12 *May*. 1 roe deer: 3 ♂♂, 3 ♀♀; 1 badger: no female ticks.

(iv) *High Thorneyburn*

Tick activity season and weight of sheep infestation practically the same as Lordenshaws.

1943

5–6 *May*. 3 rabbits: 2 ♀♀, 18 oo, 2 LL; 1 lapwing: 3 oo.

1945

17 *May*. 1 common frog: nil.

(v) *Parkhead and Mainsrigg*

1940

8 *June*. 1 fox cub: 1 ♂.

1946

27 *April*. 10 sheep: 396 ♀♀; 10 brown hares: 12 ♂♂, 29 ♀♀, 30 oo; 1 roe deer: 4 ♀♀.
4 *May*. 10 sheep: 408 ♀♀; 3 roe deer: 4 ♂♂, 19 ♀♀, 5 oo.

(vi) *Traquair Knowe*

Tick activity season similar to Hethpool and Lordenshaws; fairly heavy tick infestation.

1944

10–20 *April*. 9 adders: nil; 1 common lizard: nil.
20 *April*–20 *June*. 3 common lizards: nil.

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ADDENDA

(1) Apart from minor instances, we have not referred to the conclusions of Shcheglova (1939). The reason will become plain at once to anyone carefully comparing his conclusions and diagrams with the raw data in his paper.

(2) Since writing Part 1 of this paper, we have found a recent reference (LAURIE, E. M. O. (1946). *J. Anim. Ecol.* **15**, 22) to one nymph of *Ixodes ricinus* being taken from a young Coypu (*Myocastor coypus*) near Norwich. This is one more confirmation of the suggestion that the tick will parasitize any warm-blooded animal it may meet.

(*MS. received for publication* 1. IV. 1947.—Ed.)

TRYPANOSOMA UNIFORME–TRYPANOSOMA VIVAX INFECTIONS IN BOVINES AND *TRYPANOSOMA UNIFORME* INFECTIONS IN GOATS AND SHEEP AT ENTEBBE, UGANDA

By S. G. WILSON, B.Sc., Ph.D., M.R.C.V.S., *Senior Veterinary Research Officer, Entebbe*

(With 22 Figures in the Text)

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I. INTRODUCTION

In the course of recent work on the pathogenicity of *Trypanosoma vivax* in bovines, cases have occurred at the Veterinary Laboratory, Entebbe, when it has been difficult to diagnose the species of trypanosome occurring in the blood. This difficulty arose because many of the trypanosomes were too small to be typical *T. vivax* and the possible occurrence of *T. uniforme* had to be considered, and also because many abnormal degenerate forms appeared at irregular intervals. A more detailed study of the trypanosomes present in the infection was therefore undertaken and, although in many aspects this work is still incomplete, the course of the infection in goats and sheep has been sufficiently interesting to merit this preliminary report.

II. PREVIOUS RECORDS OF *TRYPANOSOMA UNIFORME* IN UGANDA

T. uniforme was first described from bovines in Uganda by Bruce, Hamerton, Bateman, Mackie & Bruce in 1911. Subsequent records have been summarized by Hoare & Broom (1938) who, after examination of three strains from Uganda and two from the Belgian Congo, formed the opinion that *T. uniforme* was a distinct species, although it only differed appreciably from *T. vivax* in the shorter mean and range of body length. The same authors (Hoare & Broom, 1939) later recorded a mixed infection of *T. vivax* and *T. uniforme* from a young situtunga from Bussi Island, Lake Victoria. From the present recorded geographical distribution, *T. uniforme* would appear to be restricted in East Africa to those areas infested by *Glossina palpalis* around the Uganda lakes and eastern parts of the Belgian Congo. The recorded hosts are cattle, goats, buffalo, bush-buck, water-buck and situtunga.

III. MATERIAL AND METHODS USED IN THE PRESENT STUDY

The material examined came from eight infected bovines with mixed *Trypanosoma uniforme* and *T. vivax* infections and two goats and two sheep, subinoculated from bovines and in which pure *T. uniforme* infections developed. Pure *T. uniforme* infections were also produced in bovines but these cases are not dealt with in the present paper.

Thick and thin blood smears were taken from infected animals early every morning, and stained in Azur II-Eosin mixture (Laws, 1931) buffered to pH 7·2–7·4. Thick smears were merely used for diagnostic purposes, but trypanosomes in thin smears were carefully identified and drawn and measured if they were sufficiently numerous. The drawing apparatus consisted of an old photo-micrographic camera on a vertical stand fitted with bellows and ground-glass focusing screen. The bellows were extended to fit over the eyepiece of a microscope, the focusing screen being fixed to give a magnification of × 2000 with a Zeiss × 20 eyepiece. For illumination, a Leitz low-voltage 6 V. 5 amp. bulb in a holder fitted with a focusing lens was used. One hundred trypanosomes were drawn if they were sufficiently numerous, but occasionally only fifty or less could be found in any one smear. The trypanosomes were then measured along an imaginary central body line with micro-dividers set at 1 mm., i.e. 0·5 μ at × 2000 magnification.

IV. MIXED NATURAL INFECTIONS IN BOVINES

Eight bovines were herded near the *Glossina palpalis* 'fly belt' at Musoli Forest, Entebbe, in August 1946, in the hope of establishing pure *Trypanosoma vivax* infections. To reinforce the infection all eight bovines were also subinoculated with blood from a pure *T. vivax* case (Bovine no. 5511) 2 weeks after exposure to *Glossina palpalis*. When blood smears from these bovines were carefully examined early in October, some showed a temporary pure *Trypanosoma uniforme* infection, while others showed what appeared to be a mixed *T. vivax* and *T. uniforme* infection, but the number and species of trypanosomes seen in the blood varied from day to day. One feature remained reasonably constant, i.e. while *T. uniforme* was often numerous in blood films, *T. vivax* was always relatively scanty or absent. The problem of establishing pure infections of both *T. vivax* and *T. uniforme* was then considered. Bovine no. 5407, one of the eight natural cases, was chosen for subinoculation experiments and the course of the infection in this animal is therefore given in brief outline. The incubation period and exact details of the early course of the disease cannot be given as this group of animals was not then under daily observation. The temperature reaction and the number of days trypanosomes were found in the blood from the 7th to 13th week after exposure to *Glossina palpalis* are shown in Fig. 1. *Trypanosoma uniforme* predominated throughout this period, but the appearance of numerous trypanosomes in the blood did not evoke any marked rise in temperature. The infection caused very little loss in weight as there was only a loss of 17 lb. during the 7-week period shown in Fig. 1.

The trypanosomes were measured on three occasions during the 10th and 11th weeks, and the result is given as a histogram in Fig. 2. On 22 October, or the 6th day of the 10th week, fifty trypanosomes were measured and ranged from 12 to 16 μ in length. Dividing forms were also numerous (Fig. 3). On the 3rd day of the 11th week (26 October 1946), eighty-five trypanosomes were measured and the body length ranged from 12 to 24 μ, and there was evidently a mixed *T. uniforme* and *T. vivax* infection present (Fig. 4). Fig. 2*b* shows how clearly the two species differ in body measurement. The single trypanosome, 18 μ long, was placed in the *T. uniforme* group. Three days later, on 29 October 1946, *T. vivax* was found scanty and *T. uniforme* was absent and ten forms measured varied from 22·5 to 25·0 μ (Fig. 5). On the following day only three *T. vivax* were found on one thin smear, and they measured 21, 22 and 23 μ, and the infection was then lost. Fuller details of bovine cases will be given in a later paper.

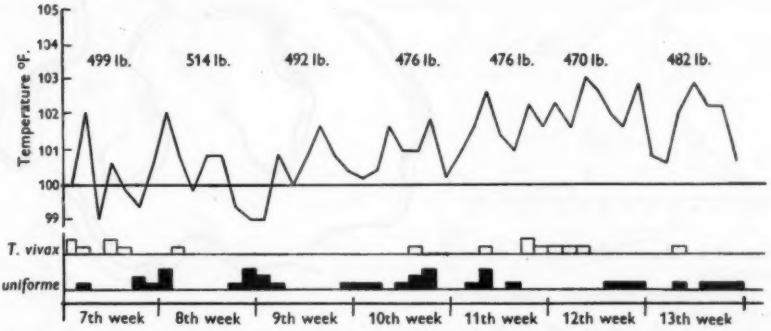


Fig. 1. Temperature reaction, body weight and appearance of trypanosomes in the blood of Bovine no. 5407 from the 7th to 13th week after exposure to *G. palpalis*.

V. PURE *TRYPANOSOMA UNIFORME* INFECTIONS IN GOATS BY SUBINOCULATION

A 3-year-old male goat, no. 2981, was subinoculated on two occasions with 10 c.c. whole blood from Bovine no. 5407, first on 11 October 1946, when *T. uniforme* was scanty in the bovine blood, and again on 21 October when *T. uniforme* was numerous. Later examination of blood smears taken on this day showed that *T. vivax* was also present but very scanty. A few *T. uniforme* were found in thick blood films from the goat on the 27th to 31st days after the first subinoculation and were first seen on thin films on the 34th day. The subsequent course of the infection from the 34th to 80th day is shown in Fig. 6. No *T. vivax* was found throughout the entire course of the disease, but *T. uniforme* appeared numerous at irregular intervals until the 79th day, after which the goat appeared to make a spontaneous recovery and the blood remained negative until the 100th day when it was discharged from the experiment.

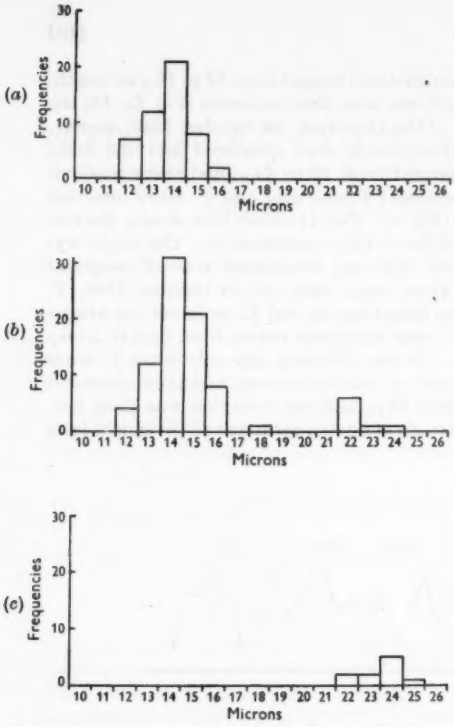
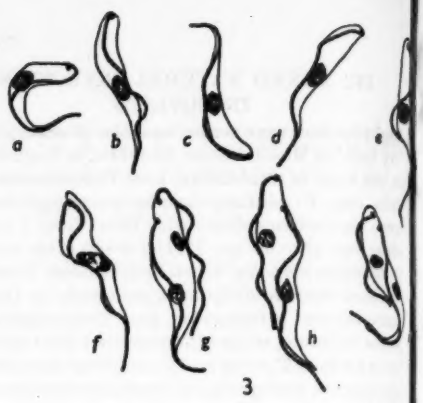
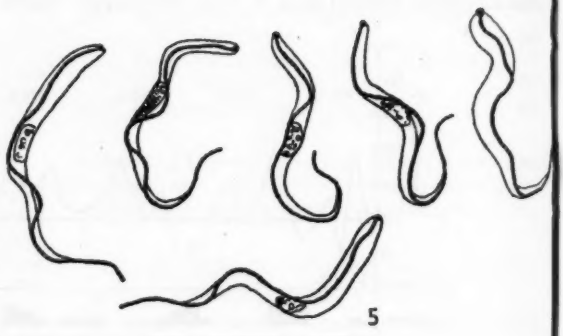
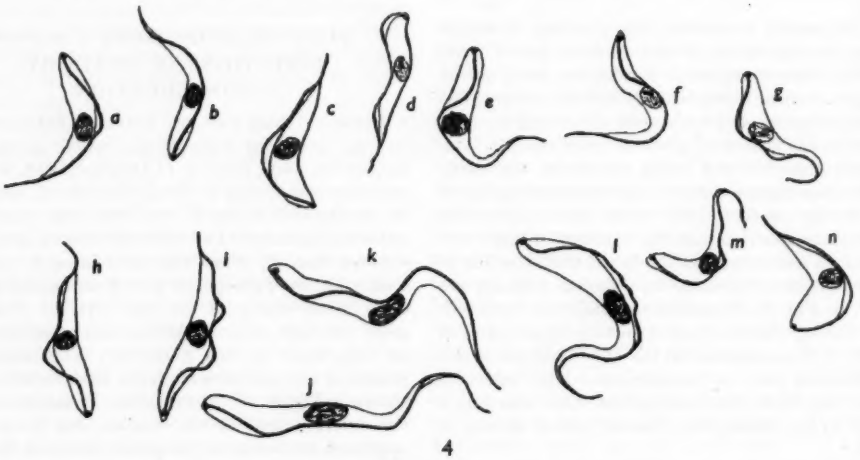


Fig. 2. Histogram of a mixed infection in Bovine no. 5407 illustrating:
(*a*) pure *Trypanosoma uniforme* infection on the 69th day;
(*b*) *T. uniforme–T. vivax* on the 73rd day; and
(*c*) a scanty *T. vivax* infection on the 76th day.







Figs. 3–5

Fig. 3. *Trypanosoma uniforme* found in blood of Bovine no. 5407 on 22 October 1946. *a–e*, normal *T. uniforme*; *f–i*, dividing forms. Fig. 4. Trypanosomes found in the blood of Bovine no. 5407 on 26 October 1946. *a–c*, *d–i* and *m* are *T. uniforme*; *j–l* are *T. vivax*; *c* and *n* are partially degenerate *T. uniforme*. Fig. 5. *T. vivax* blood of Bovine no. 5407 on 29 October 1946.

Fig.

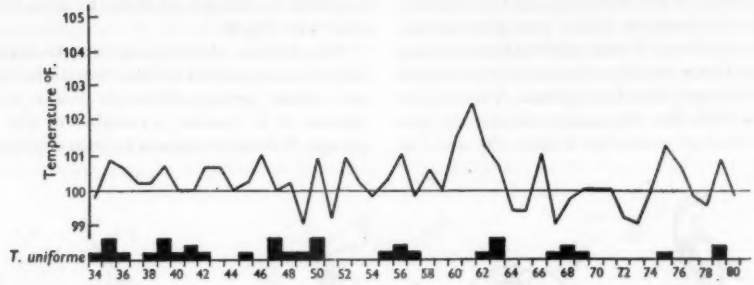


Fig. 6. Temperature reaction and appearance of *Trypanosoma uniforme* in blood of goat no. 2981 from the 34th to 80th day.

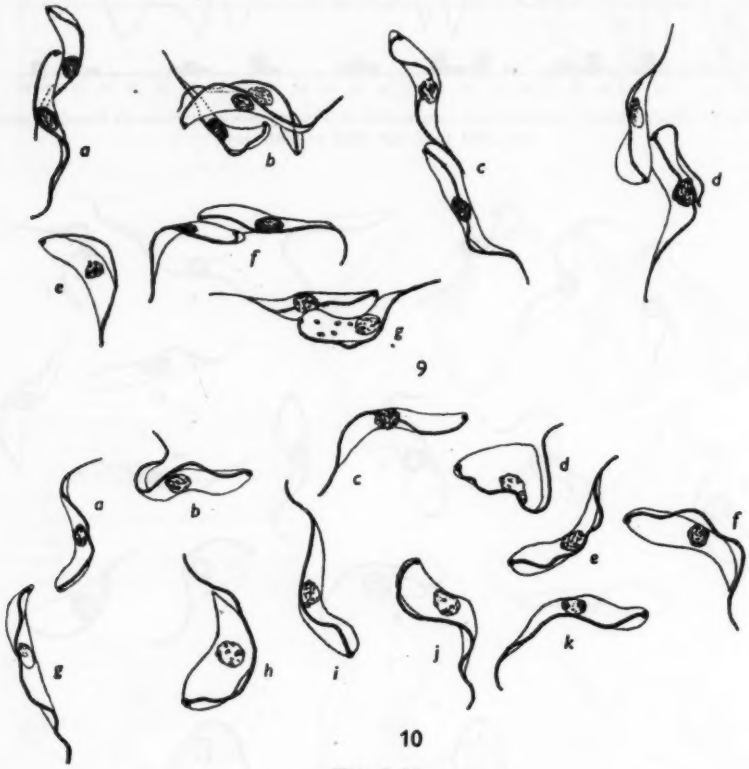
Figs. 7–8

Fig. 7. *Trypanosoma uniforme* seen in peripheral blood of goat no. 2981 at 7 a.m. on the 35th day. *g–i* and *n–q* are degenerate forms. Fig. 8. Degenerate forms seen in blood 4 hr. later on the same day.

T. uniforme was first really numerous in thin blood smears taken early on the 35th day, and a considerable number of abnormal forms was also present (Fig. 7). These abnormal forms differed from normal *T. uniforme* in being usually short and club-shaped with cloudy and vacuolated cytoplasm. The nucleus was distorted with the chromatin broken up into four or five distinct particles within the nuclear membrane, and the flagellum appeared to be breaking free from the body. In smears taken at midday on the same day these abnormal forms predominated and many grotesque shapes were seen (Fig. 8). Degeneration in some was almost complete. Degenerate forms persisted throughout the following day, but thick and thin blood smears were negative on the 37th day.

The goat quickly relapsed as on the 39th day, at 7 a.m., trypanosomes were again numerous in the blood and degenerate forms were also present. There was a tendency for these trypanosomes to lie either together in clumps of three to five, or in pairs as shown in Fig. 9.

This feature of clumping or auto-agglutination is seldom seen in this laboratory with trypanosomes of the '*vivax*' group, although it is a very common feature of *T. simiae*, a member of the '*congolense*' group. It does not appear to be in any way connected with multiplication as the arrangement is not regular and trypanosomes may lie head to tail or with their posterior ends pointing in a similar direction. Also, normal forms may be in juxtaposition with other normal forms or with degenerate forms. In Fig. 9*d* a normal and an early dividing form lie together. The appearance of trypanosomes on this day was very transient as at 11 a.m., or 4 hr. after they had been numerous, they were extremely rare in the peripheral blood. On the 40th day trypanosomes were found in thick films only, but on the 41st day normal



Figs. 9–10

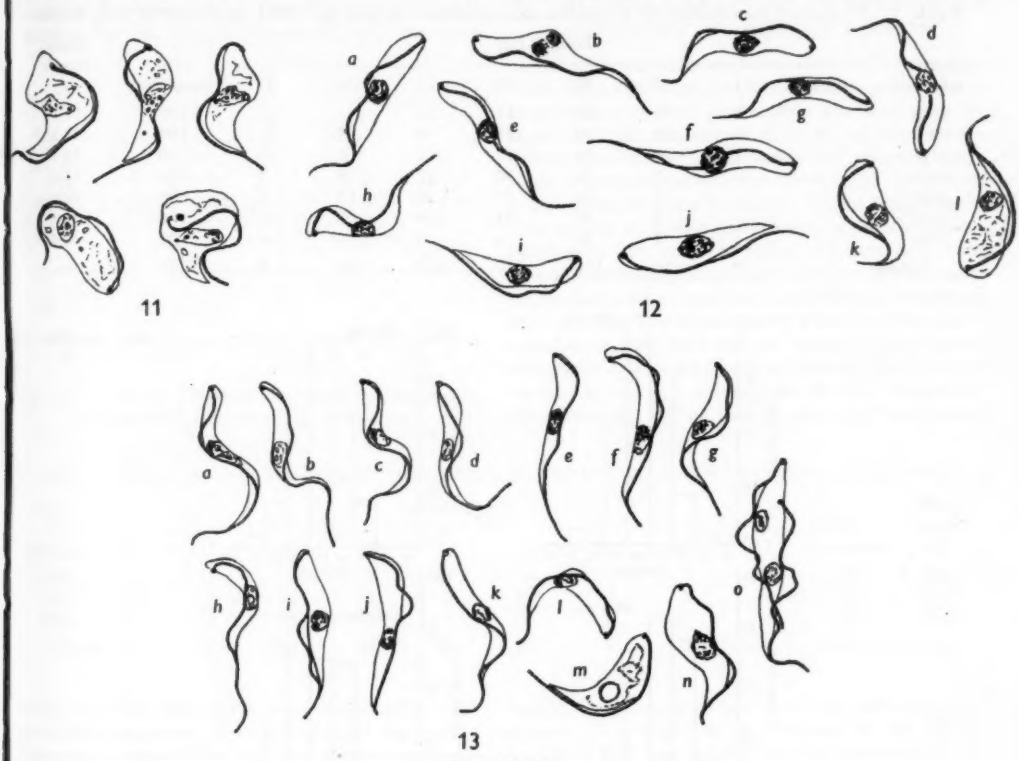
Fig. 9. *Trypanosoma uniforme* as seen in blood smears of goat no. 2981 on the morning of the 31st day of infection. Fig. 10. *T. uniforme* in goat no. 2981 at 7 a.m. on the 47th day showing normal and degenerate forms.

and dividing forms were sufficiently numerous in thin films to enable fifty normal forms to be found and drawn on one smear. On the 42nd day only three trypanosomes were found and blood was completely negative on the 43rd and 44th days. This first relapse therefore lasted for 5 days, from the 38th to 42nd day.

The second relapse occurred on the 47th day and lasted for 4 days, and trypanosomes were sufficiently numerous in the thin film to be drawn and counted on two occasions, i.e. on the 47th and 50th days. At 7 a.m. on the 47th day, *T. uniforme* were abundant, although some abnormal forms were present (Fig. 10). At noon the picture showed little change, but at 4 p.m. most of the forms seen were abnormal (Fig. 11), while at 8 p.m. all forms were rare. The resemblance of these degenerate forms to those of *T. montgomeryi* Laveran 1909 as drawn by Kinghorn & Yorke (1913) may be noted, and raises the question of the true status of this species. Trypanosomes remained very rare or negative throughout the 48th day, but showed a tendency to increase in the evening of the 49th day. On the morning of the 50th day they were again numerous (Fig. 12), but numbers present fell rapidly throughout the day and smears were negative on the 51st day.

From the 51st day onwards, the appearance of trypanosomes in the blood was very transitory and they were only really numerous on the 63rd day as shown in Fig. 13. On the 68th day trypanosomes were very rare, and on the 79th day only twenty-five forms were drawn and measured and, though numerous dividing forms were seen in the smears taken in the early morning of each day, the infection never became established.

The general trend of each relapse was very similar throughout the course of the disease. Trypanosomes would only appear numerous in the peripheral blood



Figs. 11–13

Fig. 11. Degenerate forms of *Trypanosoma uniforme* present in blood of goat no. 2981 at 4 p.m. on the 47th day. Fig. 12. *T. uniforme* as seen in blood of goat no. 2981 on the morning of the 50th day. *i–l* are in various stages of degeneration. Fig. 13. *T. uniforme* from goat no. 2981 on the morning of the 63rd day. *h* has an abnormally long flagellum while *j* has a very short one. *m* and *n* are degenerate forms while *o* is a dividing form.

for a few hours and with the appearance of abnormal forms would rapidly disappear. The first and second relapses were relatively prolonged lasting 5 and 4 days respectively with trypanosomes numerous on two of the days of each relapse. Later relapses only lasted 3, 2, or 1 day.

The trypanosomes were measured on seven different days during the course of the infection and the results are shown in Table 1 and illustrated in Fig. 14.

On the 39th day the mean length of 13·3 μ was abnormally low, but on this day many of the trypanosomes were found in clumps and others were degenerating, so there was obviously some change taking place which had caused a decrease in the numbers of trypanosomes in the 14 and 15 μ groups.

Table 1. *Frequency distribution of* Trypanosoma uniforme *in goat no.* 2981 *in respect of body length*

Day of infection	Length (μ)								No. measured	Mean length (μ)
	10	11	12	13	14	15	16	17		
35th	—	2	9	26	41	22	—	—	100	14·1
39th	1	6	28	32	21	9	3	—	100	13·3
41st	—	1	8	10	17	12	2	—	50	14·1
47th	—	—	8	24	36	22	8	2	100	14·3
50th	—	—	4	24	25	30	13	4	100	14·6
63rd	—	—	6	26	34	28	6	—	100	14·3
79th	—	—	7	10	5	3	—	—	25	13·4
Totals	1	9	70	152	179	126	32	6	575	—

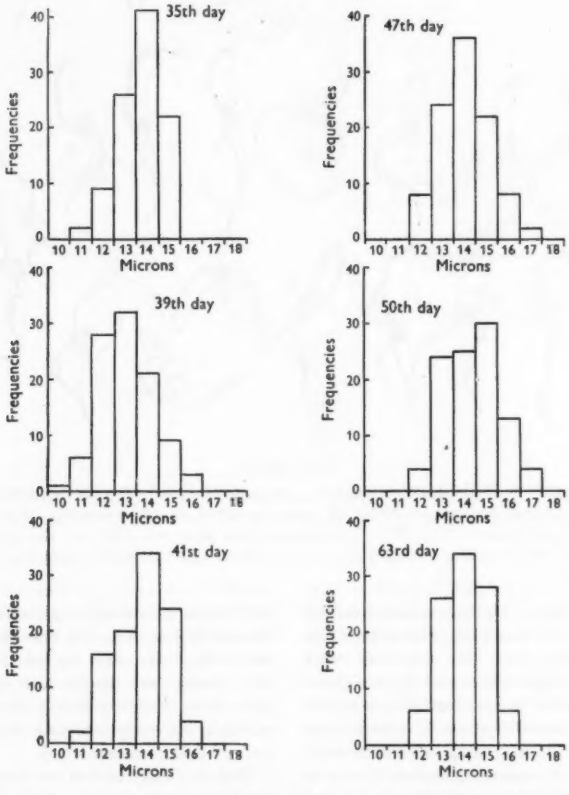


Fig. 14. Histogram showing frequency distribution in respect of length of *Trypanosoma uniforme* on six different days during the course of the infection in goat no. 2981.

When the total number of trypanosomes in each group is considered the class mode is 14 μ.

A second goat, no. 2982, 9 months old, was given subinoculations from Bovine no. 5407 similar to those given to goat 2981. In this case the incubation was short as *T. uniforme* appeared scanty on the 12th day after the first inoculation. They disappeared, however, from the blood within a few hours and did not reappear again until the 17th day. The course of the disease from the 17th to 47th day is shown in Fig. 15. On the 18th day *T. uniforme* were

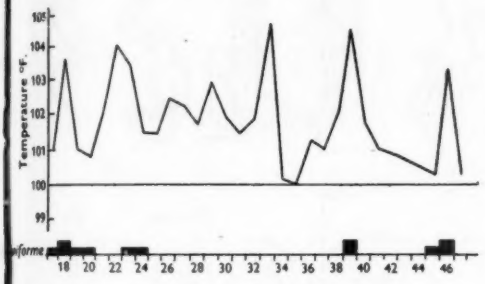


Fig. 15. Course of *Trypanosoma uniforme* disease in goat no. 2982 from the 17th to 47th day.

In Fig. 19 the mean body lengths are shown in the form of a histogram. On the 39th day degeneration or lysis of the trypanosomes had started at the time the blood smear was taken and it would appear that the 14 μ group was chiefly affected and their disappearance from the blood preceded that of the 13 and 15 μ groups.

VI. MULTIPLICATION OF *TRYPANOSOMA UNIFORME* BY BINARY FISSION

Dividing forms of *T. uniforme* were frequently seen both when trypanosomes first became numerous in the blood, and during the early hours of each relapse, and the methods of division conformed to a well-defined pattern. Trypanosomes showing early signs of division were always the longest forms seen in the blood, ranging from 15 to 17 μ in body length. The earliest sign was the movement of the kinetoplast from the usual terminal position to a subterminal and marginal position, and at the same time it lost its round or oval form and became elongated and rod-like. The kinetoplasts were formed, joined together at first by a narrow connecting strand of chromatin but soon becoming distinct, the posterior becoming the new kinetoplast for the 'daughter' trypanosome. Before this division was complete a

Table 2. *Frequency distribution of* Trypanosoma uniforme *in goat no.* 2981 *in respect of body length*

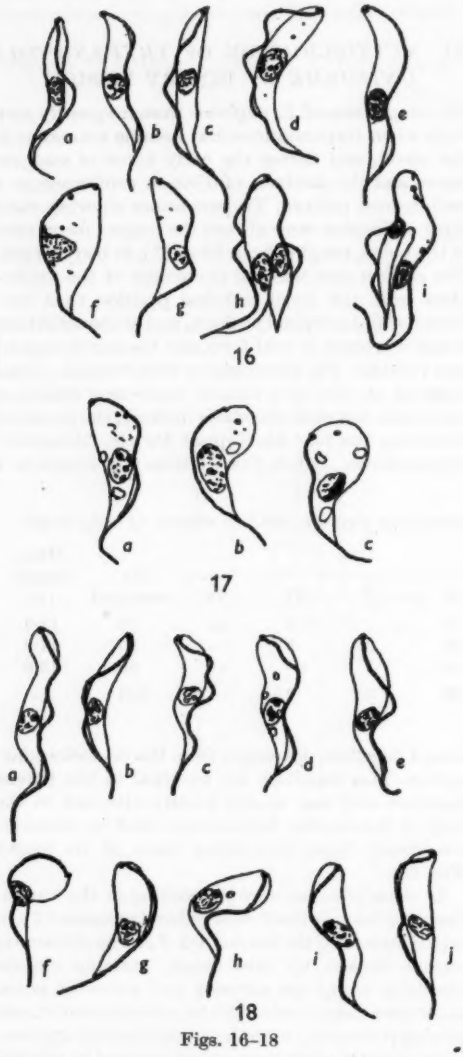
Day of infection	Length (μ) 10	11	12	13	14	15	16	17	18	No. measured	Mean length (μ)
18th	—	1	3	25	11	8	1	1	—	50	13·9
39th	—	2	9	15	5	16	2	1	—	50	14·0
46th	—	2	10	14	17	5	2	—	—	50	13·6
Totals	—	5	22	54	33	29	5	2	—	150	—

easily found in thin blood smears and fifty were drawn and measured. Both normal and degenerate forms were present (Fig. 16), but a few degenerate forms only were seen on the 19th and 20th days (Fig. 17). On the 23rd and 24th days the few *T. uniforme* found were too scanty for detailed study. No trypanosomes were found in the blood from the 25th to 38th days, but *T. uniforme* were found numerous on the morning of the 39th day. Degenerate forms were also numerous but no dividing forms were found (Fig. 18). Within 4–5 hr. all forms had disappeared from the blood. On the 46th day they were again present in sufficient numbers to be counted, but thereafter until the 100th day trypanosomes were only found scantily on 2 days and the goat was discharged from the experiment. The range and mean of body length of 150 trypanosomes measured in groups of fifty on 3 different days are given in Table 2.

second flagellum developed from the posterior kinetoplast. This flagellum lay external to the parent flagellum and was usually loosely attached to the body of the dividing trypanosome and on occasions was found lying free along most of its length (Fig. 20).

As these changes were proceeding in the kinetoplast, the nucleus itself enlarged and measured 3 μ in length instead of the normal 1·5–2 μ. The chromatin became broken up into deeply staining clumps especially along the anterior and posterior poles, and it then soon divided into two distinct nuclei, one moving posteriorly with the newly forming trypanosome while the other more or less retained its original position as the nucleus of the parent trypanosome. In the early stages of enlargement of the nucleus, before division, the original flagellum became closely connected with the nucleus and even after division may still appear to be attached to the anterior

nucleus of the parent trypanosomes. With the division of the nucleus, two distinct trypanosomes in linear arrangement could be discerned. The trypanosome moving posteriorly with the newly formed flagellum was regarded as the 'daughter' trypanosome and usually measured about 1 μ shorter than the anterior or 'parent' trypanosome. At this stage fission of the cytoplasm commenced at the anterior end (Fig. 21) and division was soon complete. In many cases the kinetoplast of the 'daughter' trypanosome remained elongated, subterminal and marginal in position for some time after division, but could be differentiated from early dividing forms by its body length of 14 μ approximately.



Figs. 16–18

Fig. 16. *Trypanosoma uniforme* in goat no. 2982 on the 18th day. A degenerate form is shown in *f*; *h* and *i* are abnormally dividing forms. Fig. 17. Degenerate forms seen in goat's blood on the 18th day. Fig. 18. Normal and degenerate forms of *T. uniforme* seen in goat no. 2982 on the 39th day.

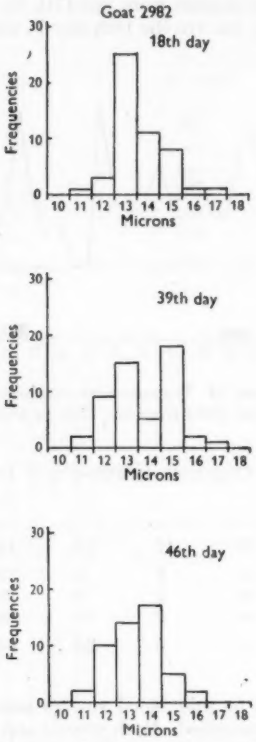
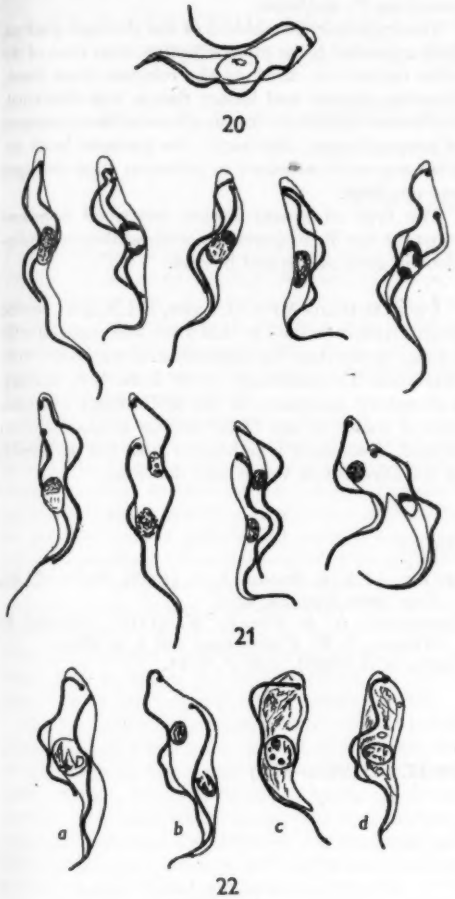


Fig. 19. Histogram showing frequency distribution in respect of length of *Trypanosoma uniforme* on three different days during the course of the infection in goat no. 2982.

Several abnormalities to this normal fission were seen. On rare occasions the kinetoplast of the 'daughter' trypanosome was seen re-dividing before fission of the cytoplasm from the parent trypanosome was complete. A fairly regular feature, seen in goat no. 2982, is shown in Fig. 16. In this case the nucleus divided *before* the kinetoplast; consequently there was no movement of the newly divided kinetoplast posteriorly and the daughter trypanosome failed to overlap the parent body. Fission of the cytoplasm from the anterior end commenced as usual soon after the division of the nucleus and the

dividing trypanosome therefore had a bifid appearance. The actual end-point of this type of division was never seen in the very numerous stained smears examined.



Figs. 20–22

Fig. 20. Early dividing trypanosomes with newly formed flagellum lying free from body. Fig. 21. Various stages of binary fission of *Trypanosoma uniforme*. Fig. 22. *T. uniforme* in sheep no. 2989 on the 31st day of infection. *a* and *b* normal dividing forms; *c* and *d* abnormal forms in which division has been arrested.

VII. *TRYPANOSOMA UNIFORME* INFECTIONS IN SHEEP BY SUBINOCULATION

Two sheep, nos. 2990 and 2989, were subinoculated with 10 c.c. whole blood from Bovine no. 5407 on 21 October 1946, i.e. when both *T. uniforme* and *T. vivax* were present in the bovine blood (Fig. 4). Sheep no. 2990, a 4-month-old female, remained negative to trypanosomiasis for 6 weeks and was then discharged from the experiment. Sheep no. 2989, an aged female, showed scanty *T. uniforme* in thick blood smears on the 11th, 12th and 19th days after subinoculation, but only a few were found in thin smears and these differed in no respect from those seen in goat blood. On the 25th and 26th days a few *T. uniforme* were again seen in both thick and thin smears. On the 30th day only two trypanosomes were found, but on the morning of the 31st day a few dividing forms were seen (Fig. 22*a*, *b*). The infection was watched throughout the day but failed to develop. At 11 a.m. a few trypanosomes, considered as early dividing forms, were seen (Fig. 22*c*), but development appeared to be arrested in that although the kinetoplast had assumed the lateral position and the nucleus was enlarged no second axoneme had developed and the cytoplasm was cloudy and vacuolated, and the posterior end of the trypanosome was abnormally swollen. The original axoneme appeared to be freeing itself from the body of the cell. At 2 p.m. all smears were negative, but at 4 p.m. a few abnormal forms were seen (Fig. 22*d*), very similar to those seen at 11 a.m. On the following day, the 32nd day, the blood was negative and scanty trypanosomes were seen on only four further occasions, i.e. on the 40th, 58th, 74th and 92nd days. The sheep was discharged from experiment on the 100th day.

VIII. DISCUSSION AND SUMMARY

Trypanosoma vivax Ziemann, 1905, *T. uniforme* Bruce, Hamerton, Bateman, Mackie & Bruce, 1911 and *T. caprae* Kleine, 1910 have often been regarded as identical or as very closely related forms. Morphologically they closely resemble each other and development in the tsetse fly occurs in the proboscis, but while *T. vivax* and *T. uniforme* are usually transmitted by *Glossina palpalis*, *Trypanosoma caprae* has been recorded from Nyasaland in exclusive *Glossina morsitans* areas (Bruce, Harvey, Hamerton, Davey & Bruce, 1913). *Trypanosoma caprae* has never been recorded from Uganda, but the present study confirms the work of Hoare & Broom (1938), and *T. uniforme* must be regarded as distinct from *T. vivax*.

A mixed *T. uniforme–T. vivax* infection in bovines is briefly described, but the work outlined in this article is chiefly concerned with the study of a pure *T. uniforme* infection in goats and sheep obtained by subinoculation from these bovines. The infection in goat 2981 was chronic and showed itself as a series of relapses and not as a fulminating or continuous blood infection. These relapses during the early weeks of infection lasted for 4–5 days, and on each occasion when trypanosomes were numerous in the blood the host reacted and the blood had a sufficiently

trypanolytic action on the circulating trypanosomes to produce disintegrating forms or even complete disappearance of the trypanosomes within a few hours. As the disease progressed the reaction of the host became more effective, and relapses were of shorter duration. This trypanolytic action produced many grotesque forms in the blood and a correct diagnosis of the trypanosomes is difficult unless the significance of their relationship with *T. uniforme* is appreciated. The average length of 575 trypanosomes measured was 14·0 μ with a range of 10–17 μ. Bruce *et al.* (1911) found that 16 μ was the average length of a total of 200 individuals measured from ox, goat and sheep, and the range was 12–19 μ. The trypanosomes in goat no. 2981 were therefore more definitely distinct from *T. vivax* than were those originally measured by Bruce. The agreement is closer with the Uganda strains H and I as described by Hoare & Broom (1938), but again there is a tendency for the present measurements to be shorter. No detailed statistical analysis of these measurements was made as the question of different strains did not arise.

In mixed infections in bovines there was always a distinct tendency for *T. uniforme* to be the dominant trypanosome, and its presence appeared to restrict the development of *T. vivax*. From the present experiments there is the possibility that when *T. vivax* was very scanty in the inoculum it failed to develop in goats owing to the presence of more numerous *T. uniforme*.

The trypanolytic reaction of the younger goat no. 2982 appeared to be more effective than that of the older animal no. 2981 as the relapses were fewer, duration shorter and binary fission was abnormal, and it was difficult to obtain accurate measurements of normal forms. Similarly, the younger lamb no. 2990 was more resistant to infection than the aged ewe no. 2989.

The type of binary fission described appeared constant for *T. uniforme* in well-established infections in goat, sheep and bovine.

I wish to thank Mr S. G. Laws, F.I.M.L.T., for the great interest he took in this work and especially for setting up the drawing apparatus on which the work depended. The assistance of Mr Lorenti S. Mukasa, Laboratory Assistant, in the preliminary examination of many of the blood smears is also acknowledged. This paper is published with the permission of the Director of Veterinary Services.

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A NEW COPEPOD PARASITE, *LERNANTHROPUS DUSSUMIERIA* N.SP., FROM THE GILLS OF A MADRAS FISH

By C. P. GNANAMUTHU, M.A., D.Sc., F.Z.S., *Director, University Zoology Laboratory, Triplicane, Madras*

(With 6 Figures in the Text)

Four specimens of this parasite were collected from the gills of about two dozen *Dussumieria acuta*, a fish which is of market value in Madras. Of the four specimens, two were female and two were male; no two were found in the same fish. Both the males were 1·5 mm. long, while the two females measured 2·6 and 2·5 mm. respectively.

Female. The cephalothoracic shield is subquadrangular, being 0·56 mm. long and about 0·64 mm. broad at its widest middle part. Its posterior edge is strongly convex so that the hindpart of the shield appears as narrow as 0·33 mm. The two lateral sides are slightly convex and folded ventrally to form lateral flaps along the entire length of the cephalothorax. The anterior edge, which extends over the antennal region, is also slightly convex. The anterolateral corners are attached to broad triangular flaps. These folded flaps being fused ventrally to the edge of the carapace, the cephalothorax appears to have oblique front corners when viewed dorsally.

The rest of the body is covered by a single dorsal plate 1·40 mm. long, being more than twice the size of the carapace. The dorsal plate is elliptical and is nearly 1 mm. at its broadest portion. It is not divided, and completely covers the four thoracic segments as well as the abdomen and anal laminae. The anterior edge extends well under the posterior convex margin of the cephalothoracic shield.

Appendages. The first antennae are inserted far apart, close to the broad anterior edge of the carapace, and extend outwards, allowing room for the second antennae to move forwards between them. The first antenna is filiform and consists of seven segments of unequal length, varying as (6/18/6/4/4/5/4), the second being longest. The outer edge of each joint is dorsally produced into a short spine extending horizontally over the next joint. The distal segment bears three pairs of slender spines, the uppermost being longest and the lowest being shortest. The second antennae are large and uncinate. Each consists of three joints, a short broad basal joint, a long stout swollen middle joint and a long sharp recurved claw-like distal joint bent inwards. The two claws curve round the gill filament of the host, which is also held lower down by the second maxillae and maxillipedes in the same manner. The second antennae, however, serve as the principal organ of prehension.

Mandibles are found within the mouth-tube. Each is a flattened structure with its oblique tip beset on the inner side by long sharp seta-like teeth. The mouth-tube has its broad base attached to the anterior side with the free-pointed apex directed backwards. The tips of the mandibles and the teeth can be made out beyond and between the upper and lower lip plates.

The first maxilla has a stout basal joint bearing a large flat transparent almost orbicular scale. The exopod consists of two joints of which the first is short and the second is more than twice its length. It bears dorsally a short spine and terminates in two longer spines, one above the other. The endopod consists of two joints and, as in the other species of the genus, is directed inwards. It is bi-articulate; the first joint is somewhat rounded, while the second is longer and connected with the mouth-tube. The second maxilla is triarticulate and uncinate. The basal joint is short and stout, the second is large and swollen at its basal portion and forms half the length of the appendage, while the third is shorter and tapers to its sharp tip which is slightly curved outward. This third claw-like joint folds down on the middle part like a knife-blade. No teeth were noticed on the inner edge of this claw. The maxillipedes form a single pair and are three-jointed. A short basal joint, a large stout swollen second joint and a tapering claw-like third joint folding on the second joint make the maxillipedes resemble the second maxillae. On the inner aspect of the second joint, just where the distal joint folds down, there is a thickened plate as in other species of this genus.

The first two free thoracic segments are very short, and succeed each other close behind the base of the maxillipedes which, therefore, usually hide the appendages of those two segments. These are very small and biramous, the two rami being inserted into

notches of the thin basal plates of the segments. The first thoracic limbs are inserted more outwards, while the second thoracic appendages are more medial in position. The exopod of the first thoracic appendage is an obovate three-jointed plate, bearing five stout sharp teeth on its outer edge, while the endopod which is inserted slightly farther is conical and bears

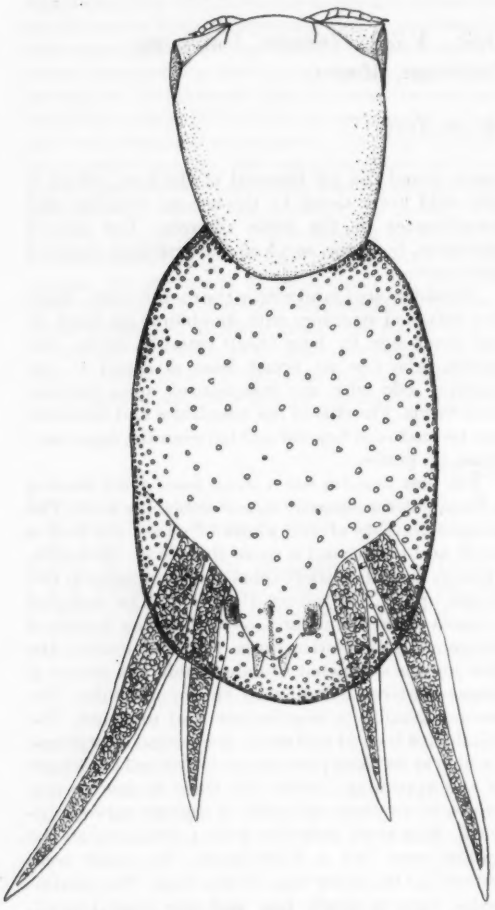


Fig. 1. *Lernanthropus dussumieria* n.sp., female. Dorsal view, × 50.

a long pointed spine. The exopod and endopod of the second thoracic leg are also inserted into notches which, however, are more close to each other. The exopod is smaller and consists of a small flat plate bearing five long teeth, while the endopod is a small conical body bearing a long spine. The third thoracic segment is much larger and is marked by foliaceous appendages characteristic of the genus *Lernanthropus*. Each appendage is formed of a basal plate with a broadly obtuse anterior tip and two broad elliptical plates attached to its posterior side. These two plates, the exopod and endopod, overlap each other and extend backward. The inner plates (the endopods) of the two sides overlap medially and effectively cover the thorax, hiding the bases of the

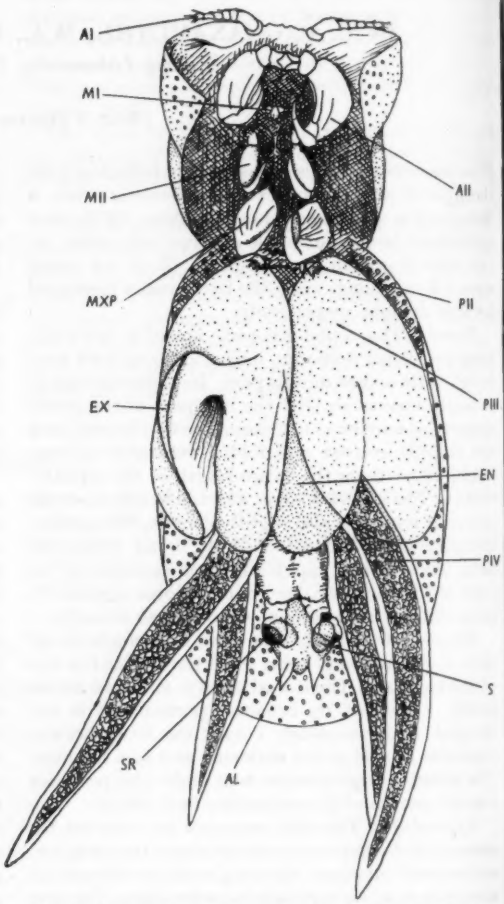


Fig. 2. *Lernanthropus dussumieria* n.sp., female. Ventral view, × 50. *A* I, first antenna; *A* II, second antenna; *AL*, anal laminae; *EN*, endopod of the third thoracic appendage; *EX*, exopod of the third thoracic appendage (turned up); *M* I, first maxilla and mouth-tube; *M* II, second maxilla; *MXP*, maxillipede; *P* II, *P* III and *P* IV, second, third and fourth pereiopods. *S*, spermatophore; *SR*, spermatophore receptacle.

fourth pair of legs as well as a considerable part of the genital segment. The fourth pair of legs has a short basal stem which bifurcates into two laminae. Of

these, the outer is stouter and longer, while the inner, which is only two-thirds as long, is covered by the dorsal plate in such a way that only a third of the length of the endopod projects behind the plate. There are no vestiges of the fifth pair of legs. The genital segment is slightly broader but far longer than the abdomen and constricts slightly at the commencement of the abdomen. The vulvae are very large and well marked. Close behind them, in one of the specimens, were large blackish brown spherical receptacles of the spermatophores. These were attached to the vulvae by the ends of the discharging tubes. The spermatophores themselves were two ellipsoidal speckled transparent plates attached to the receptacles ventrally. During the placing and removing of the cover-slips these thin plate-like spermatophores became dislodged and drifted away.

collected from *D. acuta* during the same week in December 1946 as the females were found. That these belong to the same species as the females described above is an inference based on the close resemblance in the structure of the antennae and antennules, the mandibles, the first and second maxillae, the maxillipedes, the first thoracic appendages, the genital segment and the abdomen. The conclusion is also supported by the fact that the differences are such as occur between the sexes in other species. As among the few Indian species recorded only a few males have been described, a detailed description is given here.

Both specimens found were of the same size, being 1·5 mm. long and about 1·56 mm. broad between the tips of the fourth pair of legs, though only 0·42 mm. across the cephalothorax which is the broadest part

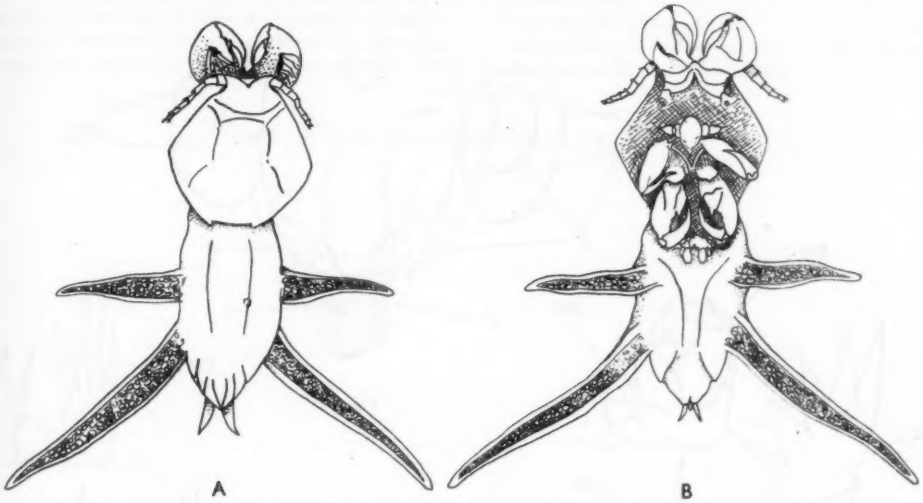


Fig. 3. *Lernanthropus dussumieria* n.sp., male. (A) dorsal view, × 40; (B) ventral view, × 40.

The abdomen is much broader than long, and carries two lanceolate anal laminae. The dorsal surface appears furrowed medially in such a way that its obtuse posterior tip, which extends dorsally over the bases of the anal laminae, appears divided. The lateral sides are so convex that the middle of the abdomen appears swollen and tapers to the narrow posterior end where the laminae are attached. The anal laminae are attached ventrally, slightly in front of the extreme posterior tip of the abdomen. They are divergent and are much shorter than the length of the abdomen, and do not reach the posterior margin of the dorsal plate. The hind-end of each lamina appears cylindrical and not only ends in a stout straight spine but also bears a stouter spine dorsally.

Male. Two male specimens of the parasite were

of the body. The cephalothorax is covered by a dorsal shield or carapace which is roughly hexagonal in form, the anterior and posterior sides being shorter than the four lateral edges. The front margin is extended over the frontal area and covers the bases of the antennae which, however, emerge on each side through notches at the two antero-lateral corners. The posterior edge of the carapace is broken by a broad shallow notch. The cephalothoracic plate is 0·43 mm. long and as broad. The rest of the body is uncovered as in all males of the genus, there being no dorsal plate.

Appendages. The second antennae are of the same form as in the female, but far larger and capable of being swung forwards. The triangular distal claw especially is longer and sharper. The triangular

plate-like reinforcement on the anterior aspect of the second joint is more conspicuous. The second maxillae and maxillipedes also are far longer and more mobile than in the female. While the limbs of the first free thoracic segment resemble those of the female, the appendages of the three thoracic segments are markedly different in the male, accentuating the sexual dimorphism indicated in the differences in size and the absence of the dorsal plate. The exopod of the second thoracic limb consists of three joints as in the female, but is of the form of a sessile club, the first two joints forming the base and the third forming the rounded end. Five teeth or broad sharp tipped plates cover the knob-like end.

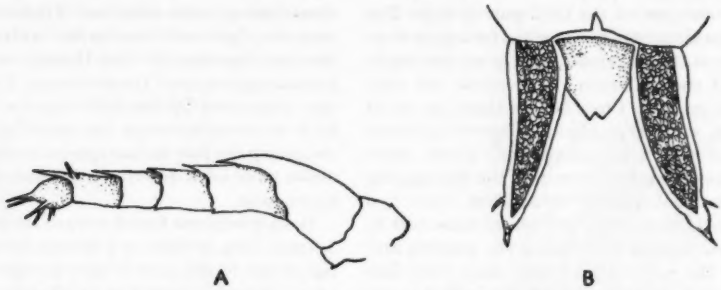


Fig. 4. *Lernanthropus dussumieria* n.sp. (A) antennae; (B) anal laminae.

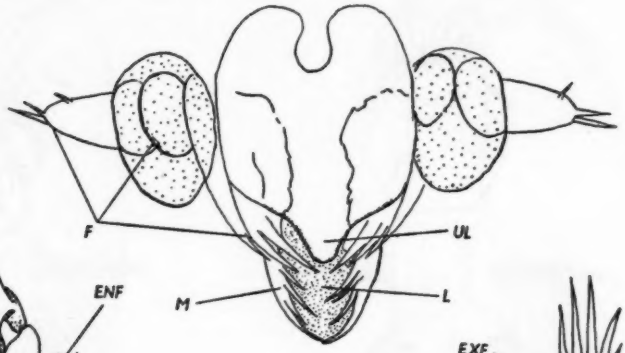


Fig. 5

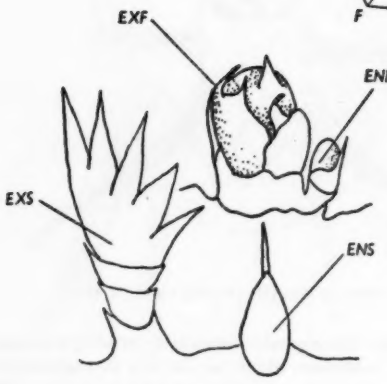


Fig. 6A

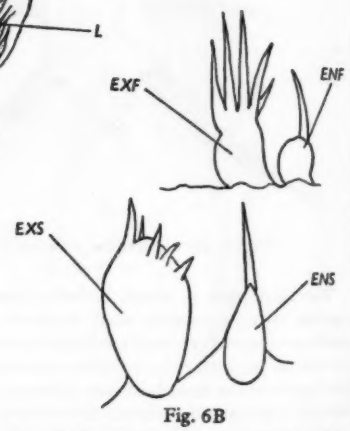


Fig. 6B

Fig. 5. *Lernanthropus dussumieria* n.sp., mouth-tube, first maxilla and mandibles. *M*, mandible; *L*, labium; *UL*, labrum; *F*, first maxilla.

Fig. 6. *Lernanthropus dussumieria* n.sp. First two thoracic appendages of (A) male, (B) female. *ENF*, endopod of first appendage; *ENS*, endopod of second appendage; *EXF*, exopod of first appendage; *EXS*, exopod of second appendage.

With the outer free edge of the second joint bearing a long bent seta, the entire exopod of this appendage accentuates the difference between the males and the females. The third thoracic limb is a uniramous cylindrical tapering process which, as in all the species of the genus, is directed outwards at right angles to the long axis of the body. The fourth thoracic appendages are also uniramous, cylindrical and tapering, but much longer and stouter. Further, they are attached so obliquely that their posterior ends extend backward, diverging greatly.

Systematics. The species to which the female and male described above belong differs from all other known species, particularly those recorded from the Indo-tropical region, in the following combination of features: the possession of a single dorsal plate by the female, the form of the cephalothoracic shield, the seven-jointed antenna bearing six spines at its tip, the narrow strip-like first and second free thoracic segments, the form of the laminae of the third and fourth legs, the form of the genital segment, the abdomen and anal laminae. Of the seven species (*Lernanthropus giganteus* Kroyer, *L. larvatus* Heller, *L. atrox* Heller, *L. nudus* Bassett Smith, *L. cornutus* Kirtisinghe, *L. pristipomidis* Kirtisinghe and *L. sciaena* Gnanamuthu) recorded from Indo-tropical regions only *L. larvatus* has a single dorsal plate but has a cephalothoracic shield longer than the dorsal plate. From the key Wilson has furnished to all the known species, one finds that the nearest forms *L. frondeus* Wilson and *L. obscurus* Wilson both have the dorsal plate distinguished by a median posterior sinus absent in the species described in this paper. Further, the carapace is widest about the middle in the present form. Hence this species (which occurs on the fish *Dussumieria acuta*) is treated as a new species *Lernanthropus dussumieria*.

Host: *Dussumieria acuta*.

Habitat: Gills.

Locality: Madras, S. India.

The holotype (♀) and allotype (♂) will be deposited in the Indian Museum, Calcutta.

(*MS. received for publication* 26. v. 1947.—Ed.)

PSEUDOPORRORCHIS TELIGER, A NEW SPECIES OF ACANTHOCEPHALA FROM JAVA

By HARLEY J. VAN CLEAVE, *Department of Zoology and Physiology, University of Illinois, Urbana*

(With 5 Figures in the Text)

Joyeux & Baer, in 1935, recognized a new genus when they described *Pseudoporrorchis houdemeri*, a new species of Acanthocephala taken from the intestine of *Centropus sinensis intermedius* at Tonkin, Indo-China. They reviewed the literature and assigned four additional species to this genus as follows: *Echinorhynchus rotundatus* von Linstow, 1897; *E. centropi* Porta, 1910; *E. bulbocaudatus* Southwell & Macfie, 1925; and *E. centropusi* Tubangui, 1933. These same authors presented a tabular comparison of the five species, based upon all of the usual morphological details utilized in formulating specific descriptions. Morphologically the five species show close conformity in all essential features. In their host relations they are equally uniform in that each species utilizes some species of the avian genus *Centropus* as its definitive host. In the present paper a sixth species of this genus is described in which the same adherence to general morphological pattern is observed, although the definitive hosts are mammals instead of the customary bird hosts.

Through the courtesy of Dr J. H. Sandground, the writer has had the privilege of examining the Acanthocephala which he collected in Java in 1938. In this material there is a fine series of worms from two different mammalian hosts (*Herpestes* and *Felis*) which show the same features recognized as distinctive for all previously recognized species of *Pseudoporrorchis*, yet they fail to agree with any known species in morphological details. Careful evaluation of these points of difference dictates that the material from mammals of Java be recognized as a distinct species which is here described as *Pseudoporrorchis teliger* n.sp.

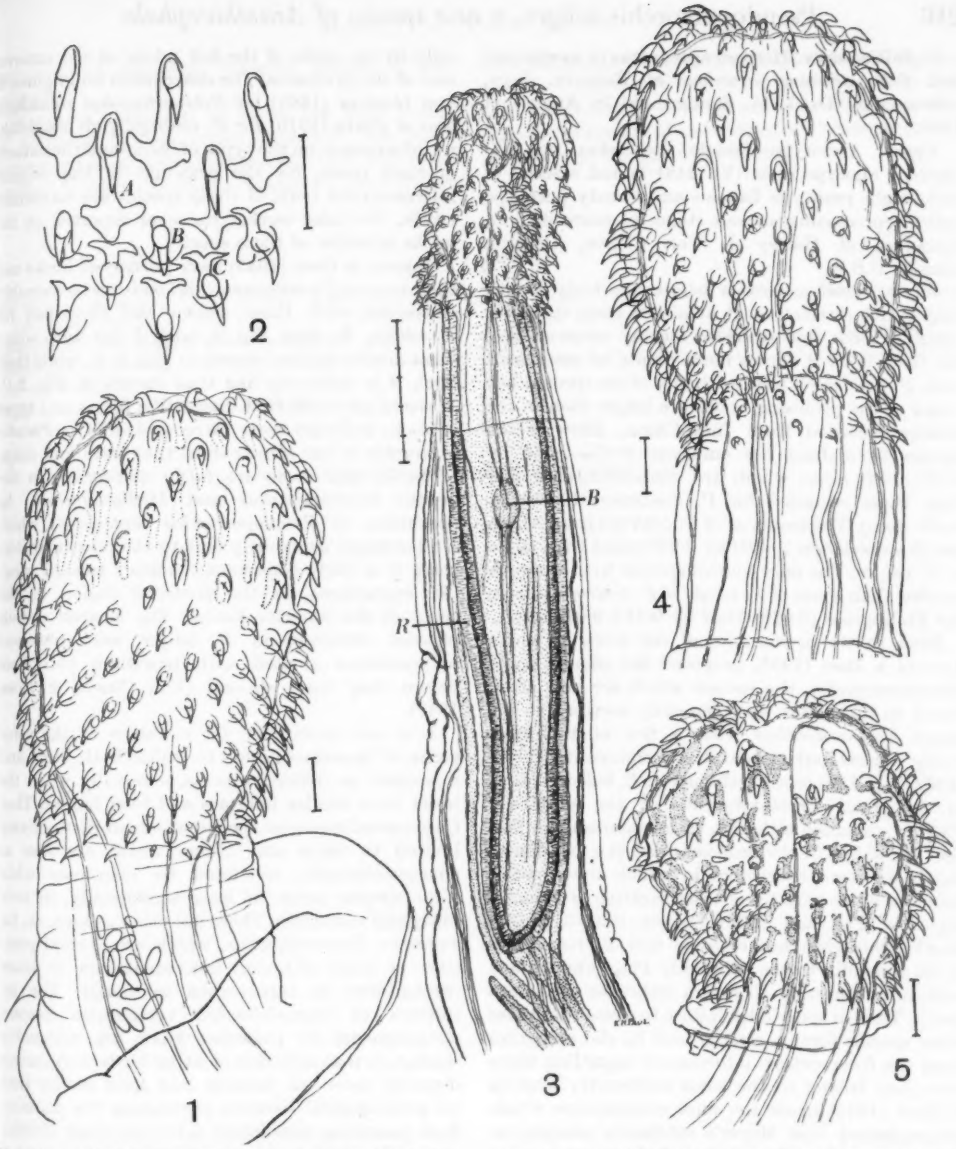
Pseudoporrorchis teliger n.sp.

(Figs. 1–5)

Specific description: With the characteristics of the genus *Pseudoporrorchis* as interpreted by Joyeux & Baer (1935). Body long, cylindrical, females reaching 48 mm., males 36 mm. Maximum diameter of mature individuals 1·6–3·1 mm. Short clavate proboscis with an ovoidal distal end somewhat larger than the base, approximately 0·75–0·86 mm. long by 0·48–0·60 mm. in maximum diameter, armed with 24–28 approximately longitudinal rows of 9 or 10 hooks each. Largest hooks each having a sickle-shaped blade with extremely long (to 0·105 mm.), narrow root, which in the second and third member of each longitudinal row frequently bears a pair of triangular wings (Fig. 2, *A*) near its posterior end. Largest hooks from 0·084 to 0·095 mm. long with a diameter of at least 0·030 mm. at the point where the blade joins the root. Hooks immediately posterior to those bearing the winged roots having peculiarly modified roots of two distinctive types. At this level the hooks of adjacent rows have roots that are wholly unlike in form. In one half of the longitudinal rows the fourth hook has a much shortened, simply expanded posterior process (Fig. 2, *B*), resembling the blade of an adze or axe. In the alternating rows, adjacent to these, the roots have a unique appearance. The root process on this type of hook (Fig. 2, *C*) is chiefly anterior to the base of the thorn while anteriorly this projection is bifurcated into two laterally spreading horns. Each hook at the base of the proboscis possesses a small, simple, disk-like root surrounding the base of its thorn.

Neck a truncated cone about 0·34 mm. long, often partially obscured (Figs. 1, 3) by its base being retracted within the front end of the trunk. Proboscis receptacle a closed cylindrical sac with uniformly rounded, somewhat pointed posterior extremity through which the invertor muscles emerge; receptacle wall (*R*, Fig. 3) composed of two heavy, concentric muscle layers. Brain (*B*) located far forward in receptacle, not far from the anterior end of the trunk; retinacula passing laterally from brain to body wall. Lemnisci somewhat longer than the receptacle, often indistinct and usually obscured by other structures, especially in the females.

Testes elongate, ellipsoidal, usually considerably separated from each other. The cement glands elongated, massed together, number and individual shapes not discernible. Mature eggs within ligament sacs of gravid females 0·053–0·059 mm. long by 0·020–0·027 mm. in width. Larval stages and intermediate hosts not known.



Figs. 1–5. *Pseudoporrorchis teliger* n.sp. All drawings were prepared by Katharine Hill Paul, scientific artist in the department of Zoology and Physiology in the University of Illinois, from stained permanent mounts. The scale accompanying Fig. 3 is 0·5 mm. long; that with each of the other drawings is 0·1 mm. long.

Fig. 1. Praesoma of holotype female from *Herpestes*, showing arrangement of the proboscis hooks. Elliptical bodies in the region of the neck are eggs.

Fig. 2. Form and arrangement of hooks on the lateral surface of the proboscis. The three chief types of hooks are indicated by *A*, *B* and *C*. Simple thorns from the base of the proboscis are not included here.

Fig. 3. Praesoma and anterior region of trunk of an immature individual from *Felis minutus javanicus* showing brain (*B*) far forward in the double-walled proboscis receptacle (*R*).

Fig. 4. Proboscis and neck of a mature individual from *Herpestes*. Note that the hooks are arranged more nearly in straight longitudinal rows than in Fig. 1.

Fig. 5. Partially introverted proboscis of an immature individual from *Felis*, stained to show structure of the roots of the proboscis hooks.

Definitive hosts: Herpestes javanicus (a mongoose) and *Felis minutus javanicus* at Batavia, Java, collected by Dr J. H. Sandground in August of 1938.

Types: Holotype female (accession no. VC 3482·1); allotype male (VC 3481·1) and a series of forty-eight paratype females and twenty paratype males representing various stages of maturity. In collection of Harley J. Van Cleave, Urbana, Illinois, U.S.A.

While *Pseudoporrorchis teliger* has body length that is not distinctive, it attains a body diameter which exceeds that recorded for any other species. On the whole, it shows more points of agreement with *P. houdemeri* than with any other species, but many of the proboscis hooks are larger than in the species recorded from Indo-China; likewise the species from Java has embryos (0·053–0·059 by 0·020–0·029 mm.) which are considerably smaller than those recorded for *P. houdemeri* (0·070 by 0·031 mm.). The embryos of *P. centropi* from Africa are likewise larger (0·080 by 0·030 mm.) than those of *P. teliger*. The only known species with embryos smaller than those of *P. teliger* is *P. centropusi* from the Philippines (0·029–0·037 by 0·015–0·019 mm.).

Prior to the appearance of the work in which Joyeux & Baer (1935) proposed the generic name *Pseudoporrorchis*, the species which are now attributed to this genus were generally ascribed to the genus *Echinorhynchus* with a few of the more recent writers making tentative assignments. Meyer (1932–3, p. 130) tentatively placed *E. bulbocaudatus* in the genus *Prosthorhynchus* of the subfamily Plagiorhynchinae although he recognized the fact that the shape of the proboscis and the location of the brain were characters in which that species failed to agree with his characterization of *Prosthorhynchus*. The same author (Meyer, 1932–3, p. 136) listed *Echinorhynchus centropi* and *E. rotundatus* in an appendix to the subfamily Plagiorhynchinae without attempting to assign either to a definite genus. Thus in spite of his failure to sense that these three species form a generic unit he did recognize from the fragmentary information regarding them that they belong to the same subfamily. Joyeux & Baer (1935) made the final comparisons which demonstrated that Meyer's subfamily assignment was correct when they gathered the species under one generic name.

In many specimens of *Pseudoporrorchis*, even some of *P. teliger*, the roots of the proboscis hooks show little axial differentiation. It was obviously on mounts of this sort that Southwell and Macfie (1925) observed for *P. bulbocaudatus* that 'Each hook is provided with a conspicuous, long, rectangular root, slightly hollowed out at its posterior extremity'. Even on superficial examination of average specimens, this description would apply only to the roots of the few hooks at the anterior end of the proboscis. The description and figures of von Linstow (1897) for *Echinorhynchus rotundatus* and of Porta (1910) for *E. centropi* give absolutely no information on the types of hooks and the natures of their roots, for the drawings in the original references for both of these species are extremely crude. No later worker has ever reported on the hooks of either of these species.

Joyeux & Baer (1935) have portrayed hooks and their roots in *Pseudoporrorchis houdemeri* reasonably consistent with those shown and described for *P. teliger*. In their Fig. 2, hook 4 has form somewhat similar to that shown in Fig. 2, *A*, while their hook 9 is distinctly like that shown in Fig. 2, *C*. It seems probable that distinctive forms and types of hooks and their roots are recognizable for *Pseudoporrorchis* to the same extent that form and shape of hooks and roots are fairly distinctive in the genera *Centrorhynchus* and *Mediorhynchus*. In specimens of *Pseudoporrorchis centropusi*, which Dr Tubangui generously sent to the present writer, there is a fairly consistent tendency toward wing-like expansions on the posterior region of the roots of the anterior hooks. The clearest details of root structure in *P. teliger* were observed in specimens treated with trisodium phosphate before they were stained (Van Cleave & Ross, 1947).

It is not uncommon for members of the same genus of Acanthocephala to utilize both birds and mammals as definitive hosts, especially when the hosts have similar habitats and food habits. Thus *Corynosoma* has some species which are distinctively limited to birds and others which are just as characteristically restricted to mammals while some species occur, at least occasionally, in both birds and mammals. There are other genera, as, for example, *Centrorhynchus*, which are normal parasites of birds although individuals are at times encountered in carnivorous mammals. The intestines of warm-blooded vertebrates present environments for parasites which are reasonably similar, so that infection of either birds or mammals depends more on habitat and food habits than on physiological barriers preventing the parasites from becoming established in the members of either group. The high incidence of individual host infection found for *Pseudoporrorchis teliger* suggests the probability that second intermediate or reservoir hosts may intervene in the life cycle to pyramid the individual host infection. Snakes and amphibians are well known as second intermediate hosts for the family Centrorhynchidae which includes the genus *Pseudoporrorchis*. The proverbial use of snakes in the diet of the mongoose makes it seem particularly probable that the mongoose obtains its *Pseudoporrorchis* by eating snakes, lizards or amphibians

carrying the encysted juvenile stages of the parasite.

In general treatises on birds, the food habits of members of the genus *Centropus* are often generalized as consisting of insects, lizards and small snakes. It seems probable, therefore, that similar food habits may result in *Pseudoporrorchis* becoming established in either avian or mammalian hosts and that the host differences do not offer any obstacle to the interpretation of the species of *Pseudoporrorchis* as a natural taxonomic grouping.

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A LIST OF THE TRICHOSTRONGYLID LUNGWORMS (PHYLUM NEMATODA) AND A KEY TO THE SIX GENERA

By ELLSWORTH C. DOUGHERTY, Ph.D., M.D.
*Division of Medical Physics, University of California, Berkeley, California**

(With 2 Figures in the Text)

In recent papers I have given, in relatively compact form, lists of the genera and species of metastrongylid lungworms; these publications are in turn summarized in a paper which follows (Dougherty, 1948). However, the trichostrongylid lungworms, constituting the subfamily Skrjabingylinae Skriabin, 1933, have not been completely treated, although in a recent work (Dougherty, 1945), in which certain lungworms were formally transferred from the Metastrongylidae to the Trichostrongylidae, the Skrjabingylin genera were listed without critical appraisal, and in the same paper the genus *Crenosoma* Molin, 1861, was reviewed in detail; subsequently the genus *Dictyocaulus* Railliet & Henry, 1907, has also been reviewed (by Dougherty, 1946).

It is desirable, I feel, to have a single list of the lungworm genera and species of the family Trichostrongylidae Leiper, 1912, particularly because of their long, erroneous association with the family Metastrongylidae Leiper, [1909], and also because in my recent paper (Dougherty, 1945), in which the systematic position of these forms was clarified, there were errors in both of the listings made of Skrjabingylin genera (pp. 57, 58), especially the inclusion of the genus *Heterostrongylus* Travassos, 1925, which is actually metastrongylid. Aside from the species *Crenosoma* and *Dictyocaulus* I have not summarized those of the Skrjabingylinae.

The six genera and twenty-one species of the trichostrongylid lungworms are listed here, together with the type species of the genera and type hosts of the species. No new synonymies and but one new combination are involved. The list is followed by a key to the acceptable Skrjabingylin genera, which serves to indicate the critical generic criteria.

Figs. 1 and 2 demonstrate the trichostrongylid nature of the ovejectoral apparatus in the genus *Dictyocaulus* and the genus *Skrjabingylus* Petrov, 1927. It can be seen that in these forms the ovejectors closely resemble those illustrated for the genus *Crenosoma* in my earlier paper (Dougherty, 1945) and are quite different in structure from those found in the Metastrongylidae *sensu vero* (Dougherty, 1948).

* Address in January, 1948: Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena 4, California.

LIST OF THE TRICHOSTRONGYLID LUNGWORMS (SUBFAMILY SKRJABINGYLINAE)

Subfamily Skrjabingylinae Skriabin, 1933

Type genus: **Skrjabingylus** Petrov, 1927

Genera (6):

Crenosoma Molin, 1861 (see Dougherty, 1945).

Genotype: *Crenosoma vulpis* (Dujardin, [1844]) Railliet, 1915 (by subsequent designation: Yorke & Maplestone, 1926).

Species (9):

Crenosoma vulpis† (Dujardin, [1844]) Railliet, 1915.

Type host: Western European fox, *Vulpes vulpes crucigera* (Bechstein).

† In my recent review (Dougherty, 1945) of the genus *Crenosoma* I cited a paper by Petrov & Gagarin (1938) in the list of references, but did not refer to it in the text. Inadvertently I failed to state in my discussion of *C. vulpis* that these authors were the first to record the parasite in question from the Ussurian racoon dog (*Nyctereutes procyonoides ussuriensis*). It should also be noted that the transliterated Russian title in the citation was in part incorrect because I had not been able to see the original; it also had several typographical errors. The correct title is: 'Prizheznennaia diferentsial'naia diagnostika legochnykh gel'mintozov pushnykh zverei.' I have recently obtained Petrov & Gagarin's paper on microfilm through the courtesy of Dr W. A. Pool, Director of the Imperial Bureau of Animal Health, Weybridge, Surrey, England.

Crenosoma mephitidis Hobmaier, 1941.
*Type host**: Western striped skunk, *Mephitis mephitis occidentalis* Baird.
Crenosoma microbursa Wallace, 1941.
Type host: Hudsonian striped skunk, *Mephitis mephitis hudsonica* Richardson.

Crenosoma skrjabini Pologentsev, 1935.
Type host: Common shrew, *Sorex araneus araneus* Linné.
Crenosoma striatum (Zeder, 1800) Molin, 1861.
Type host: Common hedgehog, *Erinaceus europaeus europaeus* Linné.

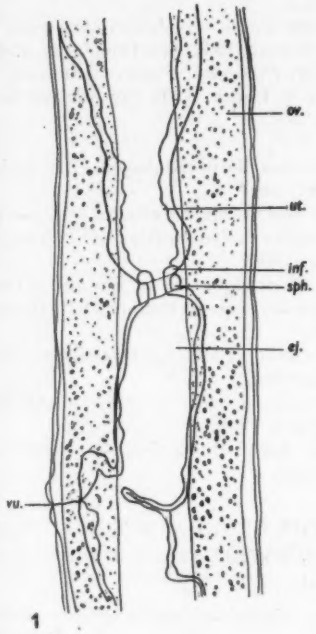

Fig. 1. Mid-region of a female of the species *Dictyocaulus filaria* (Rudolphi, 1809) Railliet & Henry, 1907, showing the ovejectoral apparatus. Original. *ej.* ejector; *inf.* infundibulum; *ov.* ovary; *sph.* sphincter; *ut.* uterus; *vu.* vulva. [*inf.* + *sph.* + *ej.* = ovejector.]

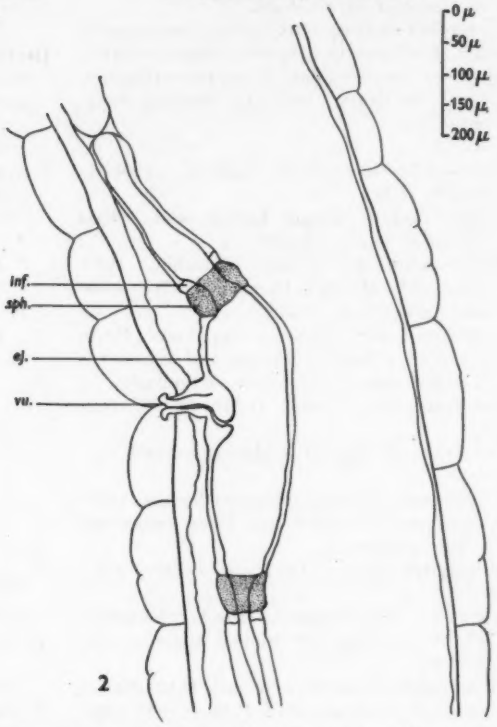

Fig. 2. As in Fig. 1, for *Skrjabingylus chitwoodorum* Hill, 1939. Original.

Figs. 1 and 2 drawn with the aid of the camera lucida.

Crenosoma petrovi Morozov, 1939.
Type host: Russian pine marten, *Martes martes ruthena* Ognev.
Crenosoma potos Buckley, 1930.
Type host: Kinkajou, *Potos flavus* (Schreber) (? subsp.).

* Hobmaier (1941 *a*) did not designate a type host and reported this species both from *Mephitis mephitis occidentalis* and from the California spotted skunk, *Spilogale gracilis phenax* Merriam. The type specimens were not designated or saved. It is desirable, I believe, to have a standard host species, and I therefore select *Mephitis mephitis occidentalis* as the type host for *Crenosoma mephitidis*.

Crenosoma taiga Skriabin & Petrov, 1928.
Type host: Kolinsky, *Mustela sibirica* Pallas.
Crenosoma goblei Dougherty, 1945.
Type host: California racoon, *Procyon lotor psora* Gray.

Bronchostrongylus Cameron, 1931 (see Cameron 1931).
GENOTYPE: *Bronchostrongylus subcrenatus* (Railliet & Henry, 1913) Cameron, 1931 (by original designation).
SPECIES (2):
Bronchostrongylus subcrenatus (Railliet & Henry, 1913) Cameron, 1931 (see Railliet & Henry, 1913).

Type host: West African leopard, *Panthera pardus leopardus* (Schreber).

Bronchostrongylus delicatus (Travassos, 1946) n.comb. (syn. *Troglostrongylus delicatus* Travassos, 1946) (see Travassos, 1946).

Type host: Golden opossum, *Didelphis marsupialis aurita* Wied.

Otostrongylus de Bruyn, 1933 (syn. *Kutassicaulus* Skriabin, 1933) (see Dougherty & Herman, 1947).

GENOTYPE: *Otostrongylus circumlitus* (Railliet, 1899) de Bruyn, 1933 (by original designation).

SPECIES (2):

Otostrongylus circumlitus (Railliet, 1899) de Bruyn, 1933.

Type host: Common harbor seal, *Phoca vitulina vitulina* Linné.

Otostrongylus andreewae (Skriabin, 1933, emend. Dougherty & Herman, 1947) Schuurmans Stekhoven, 1935.

Syntype hosts:* Okhotsk ringed seal, *Phoca hispida ochotensis* Pallas, and White Sea ringed seal, *P. h. pomororum* Smirnov.

Troglostrongylus Vevers, 1923† (see Vevers, 1923).

GENOTYPE (by original designation) and only species:

Troglostrongylus troglostrongylus Vevers, 1923.

Type host: Leopard cat, *Felis bengalensis* Kerr (? subsp.).

Skrjabingylus Petrov, 1927 (see Petrov, 1927, 1928).

GENOTYPE: *Skrjabingylus nasicola* (Leuckart, 1842) Petrov, 1927 (by original designation).

SPECIES (3):

Skrjabingylus nasicola (Leuckart, 1842) Petrov, 1927 (see Leuckart, 1942; Petrov, 1927, 1928; Baer, 1931; Swales, 1938).

Type host: Common polecat, *Mustela putorius putorius* Linné.

Skrjabingylus chitwoodorum Hill, 1939 (see Hill, 1939; Hobmaier, 1941*b*).

Type host: Louisiana striped skunk, *Mephitis mephitis mesomelas* Lichtenstein.

Skrjabingylus petrowi‡ Bazhanov, ? 1938 (see Petrov & Gagarin, 1937, 1938; Morozov, 1939).

? Syntype hosts: Common sable, *Martes zibellina zibellina* Linné, and common stone marten, *Martes foina foina* (Erxleben).

Dictyocaulus Railliet & Henry, 1907 (syn. *Bronchonema* Mönnig, 1932) (see Dougherty, 1946).

GENOTYPE: *Dictyocaulus filaria* (Rudolphi, 1809) Railliet & Henry, 1907 (by original designation).

SPECIES (4):

Dictyocaulus filaria (Rudolphi, 1809) Railliet & Henry, 1907.

Type host: Domestic sheep, *Ovis aries* Linné.

Dictyocaulus viviparus (Bloch, 1782) Railliet & Henry, 1907.

Type host: Domestic ox, *Bos taurus* Linné.

Dictyocaulus magnus (Mönnig, 1932) Dougherty, 1946.

Type host: Blesbok, *Damaliscus albifrons* (Burchell).

Dictyocaulus arnfieldi (Cobbold, 1884) Railliet & Henry, 1907.

Type host: Domestic ass, *Equus asinus* Linné.

KEY TO THE GENERA OF THE SUBFAMILY SKRJABINGYLINAE (*based primarily on male characters*)

1. Dorsal ray divided into two independent stalks. **Dictyocaulus**
   Dorsal ray a single stalk, or lacking. 2
2. Reduced (leptoderan) bursa with dorsal ray lacking. **Skrjabingylus**
   Normal (peloderan) bursa with full complement of rays 3
3. Body cuticle thrown into longitudinally striated annulations, each overlapping the next more posterior. **Crenosoma**
   Body cuticle not thrown into striated annulations. 4
4. Spicules relatively thick with non-striated laminae. **Otostrongylus**
   Spicules relatively thin, with striated laminae. 5
5. Externolateral and mediolateral rays largely fused on either side, posterolaterals arising independently. **Troglostrongylus**
   Externolateral rays arising independently, mediolaterals and posterolaterals partly fused on either side. **Bronchostrongylus**

* It would be desirable to have a designated type host. Without exact knowledge of the type material, however, I do not feel justified in making a selection.

† This date is generally given incorrectly as 1922.

‡ I have found no description of this species to date. According to Morozov (1939) Bazhanov's original description (?1938) appeared in vol. III of *Trudy Vsesoiuz. Inst. Gel'mintol.* 1938; but this has proved incorrect. *Skrjabingylus petrowi* was discussed but not described in that issue by Petrov & Gagarin (1938).

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* Reference from Morozov (1939); see footnote on p. 220.

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THE PHYLOGENY OF THE NEMATODE FAMILY METASTRONGYLIDAE LEIPER, [1909]: A CORRELATION OF HOST AND SYMBIOTE* EVOLUTION†

By ELLSWORTH C. DOUGHERTY, Ph.D., M.D.
Division of Medical Physics, University of California, Berkeley, California‡

(With 27 Figures in the Text)

I. INTRODUCTION

The family Metastrongylidae Leiper, [1909], consists of some twenty-two known genera that fall, according to my recent classification (Dougherty, 1946*b*), into four subfamilies: Metastrongylinae Railliet & Henry, 1909 (see Dougherty, 1944*a*); Filaroidinae Skriabin, 1933 (see Dougherty, 1943*b*, 1946*a*; Dougherty & Herman, 1947); Pseudaliinae Railliet & Henry, 1909 (see Dougherty, 1943*a*, *c*, 1944*b*); and Protostrongylinae Kamenskiĭ, 1905 (see Goble & Dougherty, 1943; Dougherty, 1945*a*; Dougherty & Goble, 1946). The metastrongylids are meiostomatous strongylines that live in the respiratory and circulatory system of mammals, both marsupial and placentate.

The purposes of this paper are: (1) to summarize briefly the historical treatment of the metastrongylids; (2) to present a theory on the phylogeny of the Metastrongylidae in relationship to their hosts; and (3) to give the bases for my classification of the family into subfamilies, with lists of the component genera and with the assignment of the hitherto neglected and misplaced genus *Heterostrongylus* Travassos, 1925; for treatment of species it will be necessary to consult the papers indicated in the preceding paragraph.

II. HISTORICAL SUMMARY OF THE CLASSIFICATION OF THE METASTRONGYLIDAE

Diesing (1851) was the first to group the strongylines together on the basis of the bursa of the male—as a family Strongylidea (=Strongylidae Baird, 1853§). Over 30 years later Railliet (1885) separated the meiostomes of the gastro-intestinal and respiratory tracts from other strongylines as a new subfamily Strongylinae of the family Strongylidae; during most of the nineteenth century the meiostomes were incorrectly referred to the genus *Strongylus* Müller, 1780. Kamenskiĭ (1905) was the first to subdivide them further. He recognized new subfamilies Protostrongylinae and Blastostrongylinae, the latter without a named type genus; both groups were for lungworms and in their composition correspond in my classification to the Metastrongylidae and Skrjabingylinae respectively. Since he did not assign a name to a group for the gastro-intestinal meiostomes, it may be assumed that he meant to leave them as the Strongylinae.

After *Strongylus* had been correctly restricted to a eustomatous genus by Stiles (1903), Railliet & Henry in a series of papers reorganized the strongylines and named many meiostomatous genera. They recognized (Railliet & Henry, 1909) two new meiostomatous subfamilies in the Strongylidae: Metastrongylinae and Pseudaliinae, which together corresponded to Kamenskiĭ's subfamily Protostrongylinae. The pseudaliins of Railliet & Henry constitute essentially the same group as recognized to-day—the lungworms of porpoises, or toothed whales (suborder Odontoceti); their degenerate nature long obscured their close relationship to the rest of the modern Metastrongylidae.

Leiper (1909) was the first to accord the meiostomes full familial rank—as the family Metastrongylidae of an unnamed higher strongyloid group. Within the Metastrongylidae he recognized two subfamilies—Metastrongylinae and Trichostrongylinae, on the basis of female reproductive characters. I have recently (Dougherty, 1945*b*

* In the etymologically and historically correct sense of the word—signifying an organism living in an intimate relation with another organism, the host, from which it derives protection, or nutrition, or both. *Symbiosis* thus includes *commensalism*, *parasitism*, and *mutualism* (=symbiosis of many authors).

† This work was begun in the Department of Zoology, University of California, Berkeley.

‡ Address in January 1948: Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena 4, California.

§ Baird (1853), however, though forming the orthographically correct name Strongylidae, employed the earlier classification of Dujardin (1844), who scattered the strongylines in several families.

pointed out that the criteria used by him are fundamental features and necessitate the removal of certain meiostomatous genera from the Metastrongylidae to the Trichostrongylidae as a subfamily Skrjabingylinae. The basis of Leiper's treatment of the meiostomes has been almost entirely ignored by subsequent workers and abandoned by the former himself. Not only did he recognize these fundamental features, but also the fact that the metastrongylids of porpoises properly belong with their close relatives in terrestrial mammals. In his Metastrongylinae he included the genera: *Metastrongylus* Molin, 1861; *Protostrongylus* Kamenskiĭ, 1905; *Pseudalius* Dujardin, [1844]; and *Pharurus* Leuckart, 1848.

Railliet & Henry (1912), however, reiterated their separation of Metastrongylinae and Pseudaliinae as subfamilies co-ordinate with their eustomatous subfamily Ankylostominae Railliet & Henry, 1909, and with the meiostomatous subfamily Trichostrongylinae Leiper, [1909]. Furthermore, they included within the Metastrongylinae certain lungworm genera that are fundamentally trichostrongylid in structure. Thus this subfamily (and later family) essentially became a dumping ground for all the meiostomatous lungworms and has remained so until recently.

Railliet (1916) later raised the subfamilies Metastrongylinae and Pseudaliinae of his and Henry's classification each to the rank of full family. Subsequent workers have variously accepted them as independent (e.g. Yorke & Maplestone, 1926), or as a single family (e.g. Baylis & Daubney, 1926) in an order, suborder, or superfamily Strongyloidea Weinland, 1858, or Strongylata Railliet & Henry, 1913 (=Strongylina Pearse, 1936). Lane (1917) erected within a suborder Strongylata a new superfamily Metastrongyloidea for the meiostomes. Cram (1927) later restricted this to the lungworms and erected a superfamily Trichostrongyloidea for the gastro-intestinal meiostomes. Several modern workers have accepted these superfamilial groups (Skriabin, 1941; Chitwood & Chitwood, 1937; Chitwood, 1937; etc.). Pearse (1942) has gone so far as to raise the lungworms to a suborder Metastrongylina of an order Strongylida! On the other hand, Baylis (1936) has held this tendency toward an elaborate strongyline classification to be unjustified. In general, I tend to favour his view. My feeling is that the Metastrongylidae are best treated as a family in a suborder Strongylina.

The subdivision of the Metastrongylidae and Pseudaliidae *sensu* Railliet, 1916, has been attempted by several investigators, but in general has not given satisfactory results. Skriabin and his school have developed an elaborate system that in its most recent form (Skriabin, 1941, 1942, 1946) is as follows:

Superfamily **METASTRONGYLOIDEA**
- Family METASTRONGYLIDAE
  - Subfamily METASTRONGYLINAE
  - Subfamily CRENOSOMATINAE*
  - Subfamily SYNTHETOCAULINAE* [correctly Protostrongylinae]
  - Subfamily MÜLLERIINAE† [correctly Muelleriinae]
- Family PSEUDALIIDAE
  - Subfamily PROPSEUDALIINAE‡
  - Subfamily FILAROIDINAE†
  - Subfamily SKRJABINGYLINAE†
  - Subfamily PSEUDALIINAE
  - Subfamily STENURINAE‡

In addition, Shul'ts, Orlov & Kutas (1933) have further divided the subfamily Synthetocaulinae into subsubfamilies (Synthetocaulea, Angiostrongylea, etc.)!

However, Skriabin's system is not satisfactory in that it is based on relatively minor details and ignores other more fundamental features. He has not recognized the importance of the female genitalia, specifically of the ovejectoral apparatus (see Dougherty, 1944*b*, 1945*b*); thus trichostrongylids are to be found in both of his families Metastrongylidae and Pseudaliidae. He has used certain convergent and unimportant bursal characters to define his several 'metastrongyloid' subfamilies, which accordingly are in part untenable and in part, I believe, unnecessarily refined. In separating the families Metastrongylidae and Pseudaliidae he has placed lungworms with moderately well-developed bursae in the former and those with very much reduced bursae or without bursae in the latter. (An exception is the genus *Muellerius* Cameron, 1927, for which he has accurately recognized a close affinity to the genus *Protostrongylus* despite its reduced bursa.) As a result, in his classification, the genera *Filaroides* v. Beneden, 1858, and *Osleroides* Orlov, Davtian and Liubimov in Skriabin, 1933 (=*Metathelazia* Skinker, 1931) with bursae reduced or lacking are separated as a subfamily Filaroidinae (family Pseudaliidae) from their close relatives in his subfamily Synthetocaulinae (family Metastrongylidae).

Chitwood & Chitwood (1937), although simplifying Skriabin's classification as follows:

Superfamily **METASTRONGYLOIDEA**
- Family METASTRONGYLIDAE
- Family PSEUDALIIDAE
  - Subfamily PSEUDALIINAE
  - Subfamily FILAROIDINAE

have retained the artificial segregation of the Filaroidinae with the Pseudaliinae in a family Pseudaliidae.

* Authored by Skriabin (1933*a*).
† Authored by Skriabin (1933*b*).
‡ Authored by Skriabin (1942).

Viewing Skriabin's earlier systems (1933*a*, *b*) as over-elaborate, Böhm & Gebauer (1934) proposed the following classification:

Family METASTRONGYLIDAE
Subfamily METASTRONGYLINAE
Subfamily ANGIOSTRONGYLINAE*
Subfamily BRONCHOSTRONGYLINAE*
Subfamily PSEUDALIINAE

Although this system had the merit of simplicity and succeeded in separating by themselves several, although not all, of the trichostrongylid genera into the subfamily Bronchostrongylinae, nevertheless, it was still a heterogeneous mixture. Members of the subfamily Angiostrongylinae were characterized as short, thick worms with the vulva near the anus in contrast with the Metastrongylinae, which were characterized as long, thin worms with the vulva behind the middle of the body, usually near the anus. Actually Böhm & Gebauer's two 'angiostrongylin' genera—*Angiostrongylus* Kamenskiĭ, 1905, and *Elaphostrongylus* Cameron, 1931—are composed of long, thin species and are closely related to genera placed by them in the Metastrongylinae. In fact, the genus *Parastrongylus* Baylis, 1928, placed in the latter subfamily, is actually a synonym of *Angiostrongylus* (see Dougherty, 1946*a*)!

Extended negative analysis of the classificatory systems of Skriabin's school and of Böhm & Gebauer is not profitable here. Such points can be made better evident by a positive analysis of my own theories of metastrongylid interrelationships and of the classification derived therefrom.

III. ORIGIN OF THE METASTRONGYLIDAE

With the elimination from the Metastrongylidae of lungworms possessing the trichostrongylid ovejectoral apparatus (i.e. with tripartite *ovejector*, consisting of distal *ejector*, middle *sphincter*, and proximal *infundibulum* connecting ovejector with uterus), the metastrongylids are a homogeneous group. They are all characterized by a poorly muscularized ovejectoral apparatus, which is largely unpaired and which terminates in a vulva just anterior to the anus. It is illustrated for *Metastrongylus apri* (Gmelin, 1790) Vostokov, 1905, in Fig. 1. In a previous publication (Dougherty, 1944*b*) I applied the following terminology to the parts of the metastrongylid ovejectoral apparatus: *reservoir* to the unpaired muscularized portion, *sphincter* to each paired muscularized portion, and *infundibulum* to the connecting cells between each uterus proper and its ovejector. As a result of a subsequent comparative study of the gross structure of the female reproductive tract in the Strongylina (as yet unpublished), I believe that this terminology should be changed as follows: *vestibulum* (instead of reservoir) for the unpaired portion, and *ejector* (instead of sphincter) for each paired muscularized portion; the term *infundibulum* is retained in its original usage.

The vestibulum is essentially a vagina, but how much of it is *vagina uterina* and how much *vagina vera* cannot be stated for all forms, inasmuch as I have not yet completed histological and cytological studies of the metastrongylid ovejectoral apparatus. On the basis of gross appearance, however, the greater part of the length of the vestibulum appears to be vagina uterina. Chitwood & Chitwood (1940) have pointed out that this distinction cannot usually be made in nematodes without histological study. However, in most metastrongylids there is a sharp transition in the appearance of the vagina, from a short, distal segment to the remaining, much longer, proximal portion. The distal section corresponds in appearance to the vagina vera of those strongylins in which this structure has been studied and identified histologically (see Chitwood & Chitwood, 1940).

In addition to the ovejectoral apparatus the metastrongylids all possess six lips, or *labia*, bearing at least the inner circle of six *cephalic papillae*. The external circle is complete with ten papillae, although the submedials are approximated and the lateromedials somewhat reduced in size as compared with the mediomedials. The labia and papillae are illustrated *en face* in Fig. 2 and laterally in Fig. 3 for *Protostrongylus boughtoni* Goble & Dougherty, 1943. Other cephalic features in the Metastrongylidae are very simple. There is at most a vestigial buccal capsule, or *stoma*, without telostomatal teeth, or *onchia*.

Evidence is convincing for the origin of Strongylina from the free-living Rhabditina. Within the Strongylina the eustomatous forms show the most primitive features as adults, especially the diaphanocephalids and syngamids. The first-stage eustome larva has a stoma almost identical with that of an adult member of the primitive rhabditine genus *Rhabditis* Dujardin, [1844]. The meiostome first-stage larvae in part show a similar organization (most trichostrongylids) and in part demonstrate fusion of sections of the rhabditine stoma into a simpler structure (some trichostrongylids and all metastrongylids). Because of this the meiostomes are generally regarded as more highly evolved forms, i.e. more removed from the primitive rhabditine-like ancestor.

In both ovejectoral and cephalic papillary pattern the Metastrongylidae are different from the Trichostrongylidae. In the ovejector of the former there is no evidence of the specialized sphincter found between infundibulum and ejector in the latter. All but the most primitive trichostrongylids lack labia. In view of the common lungworm habitat it is an

* Authored by Böhm & Gebauer (1934).

attractive hypothesis that the Metastrongylidae were derived from the Trichostrongylidae through the Skrjabingylinae, but the difficulty arises thereby of explaining why the metastrongylids have labia whereas the skrjabingylins completely lack them. I am thoroughly convinced that the metastrongylid unlikely, however, because the trichostrongylids show obvious similarities to the family Ancylostomatidae Nicoll, 1927, the only other strongyline group in which there is an ovejectoral sphincter. The trichostrongylids and ancylostomatids both have equatorially placed vulvae in the majority of forms,

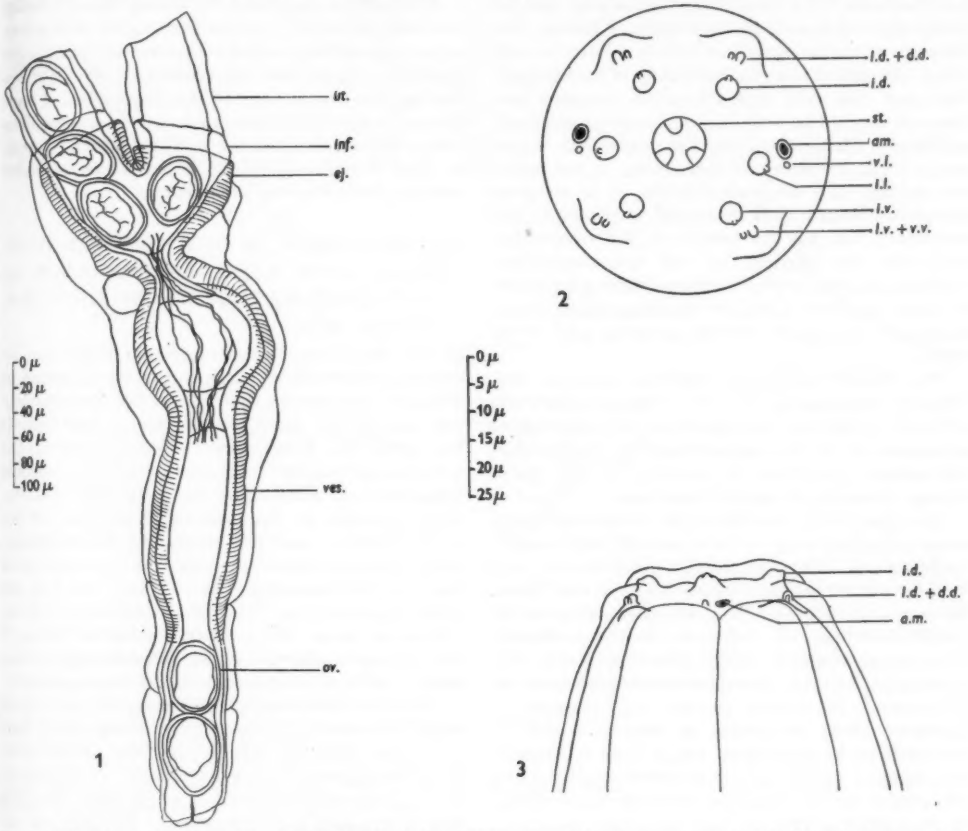


Fig. 1. *Metastrongylus apri* (Gmelin, 1790) Vostokov, 1905. Ovejectoral apparatus (original). *ej.* ejector; *inf.* infundibulum; *ov.* ovum; *ut.* uterus; *ves.* vestibulum. From camera lucida drawing.

Figs. 2–3. *Protostrongylus boughtoni* Goble & Dougherty, 1943. Anterior end (corrected after Goble & Dougherty, 1943, figs. 5 and 6).

Fig. 2. *En face* view.

Fig. 3. Lateral view.

Lettering: *am.* amphid; *i.d.* internodorsal papilla; *i.l.* internolateral papilla; *i.v.* internoventral papilla; *l.d.+d.d.* laterodorsal+dorsodorsal papillae; *l.v.+v.v.* lateroventral+ventroventral papillae; *st.* stoma; *v.l.* ventrolateral papilla.

and skrjabingylin lungworms represent convergent groups, even despite the striking similarity in the life cycle of certain members of both groups (i.e. the use of gasteropods as obligate intermediate hosts).

It is possible that the Metastrongylidae and Trichostrongylidae had a common ancestry from a primitive meiostomatous strongylin. This seems and where there has been a backward shift to a preanal condition, the tripartite ovejector has been retained. Furthermore, I believe that the equatorial position of the vulva is primitive in the Strongylina in view of their obvious derivation from the Rhabditina; this belief is in disagreement with that of Travassos (1937), who believes the preanal position

to be primitive. The trichostrongylids are in many respects ancylostomatids with vestigial stomata; in a few primitive genera of the Trichostrongylidae (e.g. *Amidostomum* Railliet & Henry, 1909, etc.) there are even fairly well-developed stomata with onchia similar to those of the Ancylostomatidae. Furthermore, the trichostrongylids largely, and the ancylostomatids entirely, lack labia. I believe that these two families are more closely related to each other than either to any other family of the Strongylina, and that they had a common ancestry in a eustome not unlike members of the ancylostomatid subfamily Globocephalinae Travassos and Vogelsang, 1932. Therefore, if meiostomy in the metastrongylids and trichostrongylids is a common ancestral feature, it is necessary to postulate the secondary loss by the former of the ovejectoral sphincter characteristic of the ancylostomatid-trichostrongylid line; furthermore, there would have to have occurred a radical rearrangement of the apparatus as a whole with the posterior shift of the vulva.

The Metastrongylidae, however, show no convincing resemblance to the Ancylostomatidae. I believe, therefore, that meiostomy is a convergent phenomenon in the metastrongylids and trichostrongylids, and that the ancestry of the Metastrongylidae is to be sought elsewhere.

The most likely candidate for a metastrongylid ancestor would seem to be a eustome with simple ovejectors (lacking sphincters), a preanal vulva, and well-developed cephalic labia. Of surviving forms those most nearly answering this description are the primitive strongylids belonging to the subfamily Cloacininae Stossich, 1899. These are the only strongylids found in Australian marsupials so far as is known. In certain genera (e.g. *Cloacina* v. Linstow, 1898) the stoma is reduced almost to meiostomy. An important change from a cloacinin to a metastrongylid condition would of necessity be the partial fusion of the long ejectors characteristic of the former group.

I believe, in view of the foregoing resemblances, that the Metastrongylidae had common ancestry with the Strongylidae in a primitive cloacinin-like ancestor. The separation of the two lines probably occurred before the mammals split into marsupial and placentate branches—in other words, in the pantotheres, or in an earlier host group. The genus *Heterostrongylus* Travassos, 1925, to be discussed in the next section, throws interesting light on this theory. In all probability the primitive cloacinins were originally gastro-intestinal symbiotes; of these, one group became secondarily adapted to a pulmonary existence and thus originated the metastrongylid line. Such an adaptation has presumably occurred at least three times in the Strongylina—once with the origin of the Metastrongylidae, once with the origin of the lung-inhabiting forms of the family Syngamidae Leiper, 1912, and once with the origin of the Skrjabingylinae. Of these the origin of the last named is probably the most recent in time. The relative time of origin of the lung-parasitism by the syngamids is, however, not clearly evident.

The outlines suggested for strongyline evolution here will be filled in more thoroughly in a subsequent paper. They bear almost no resemblance to the phylogeny postulated by Skriabin (1941) for the Strongylina and the rest of the Phasmidea. I have already pointed out certain fundamental difficulties with his theories (Dougherty, 1944*b*) as they apply to the Metastrongylidae. I leave an extended critique thereof to the later paper.

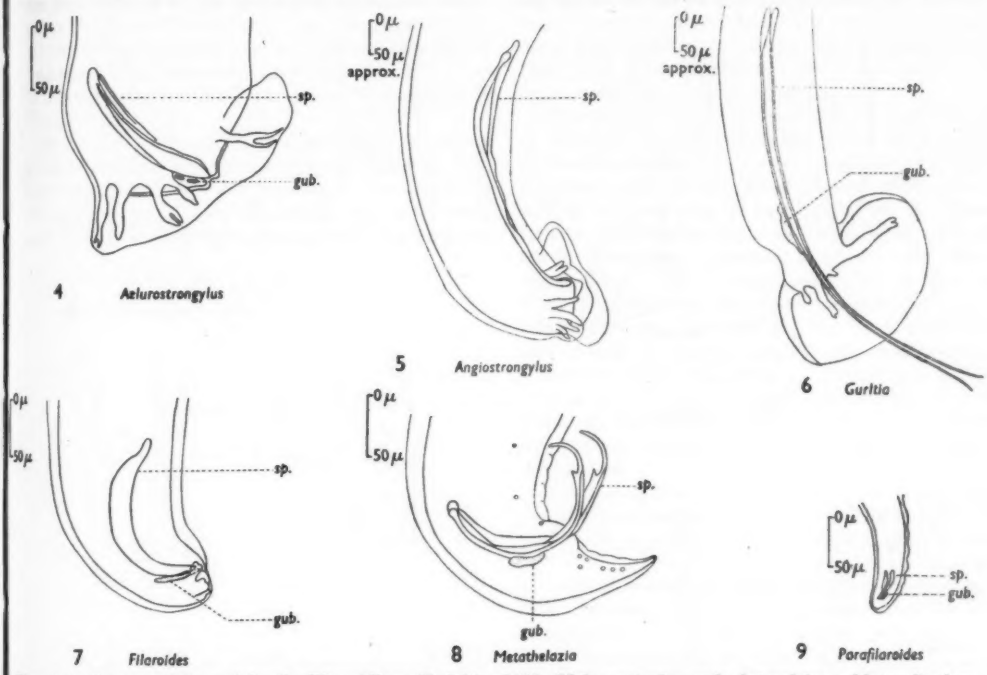
IV. RADIATION OF THE METASTRONGYLIDAE, WITH SPECIAL REFERENCE TO THE GENUS *HETEROSTRONGYLUS* TRAVASSOS, 1925

In the surviving Metastrongylidae there can be recognized three clear-cut evolutionary lines and, in addition, two genera that do not fall conveniently into any of the three groups. The former include the subfamily Filaroidinae, characteristic of the order Carnivora (although also found in the orders Primates and Rodentia); the subfamily Pseudaliinae, peculiar to the suborder Odontoceti of the order Cetacea; and the subfamily Protostrongylinae, characteristic of the suborder Ruminantia of the order Artiodactyla (although also found in the order Lagomorpha). The relict genera are *Metastrongylus* Molin, 1861, of pigs—suborder Suina of the order Artiodactyla—and *Heterostrongylus* Travassos, 1925, of an opossum—order Marsupialia.

The filaroidins and protostrongylins are particularly interesting, because on the one hand they show their ancestral host groups, but on the other hand demonstrate how it is possible for a symbiotic line to become established in a new host line. This well delineates a fundamental difficulty in an evolutionary reconstruction of a symbiotic group from its host-relationships. Where evolutionary processes have a long enough period over which to act, the pattern of origin of the symbiotic group may become completely obscured, if not by the extinction of the original host assemblage, then by the greater success of the symbiotic line in a secondarily acquired host-group.

In the case of the metastrongylid lines there is good evidence that the mammalian groups indicated have been the ancestral hosts. First of all the three orders Carnivora, Cetacea, and Artiodactyla are often considered of common phylogenetic origin (Romer, 1945, pp. 442–4, 448)—especially the Carnivora and Cetacea. Secondly, it is in these groups that the filaroidins, pseudaliins, and protostrongylins

respectively have undergone their conspicuous radiation. It seems reasonable to postulate that the three lines arose from a common ancestor symbiotizing a primitive mammal at the base of the carnivore-cetacean-artiodactyl radiation.



Figs. 4–9. Genera of the subfamily Filaroidinae Skriabin, 1933. Male posterior ends, lateral (or sublateral) view. (Figs. 4–8, as in Dougherty, 1946*a*, fig. 1; fig. 9, after Dougherty & Herman, 1947, fig. 1A.)

Fig. 4. *Aelurostrongylus fengi* (Hsü, 1935) Dougherty, 1946.
Fig. 5. *Angiostrongylus raillieti* (Travassos, 1927) Dougherty, 1946.
Fig. 6. *Gurltia paralysans* Wolffhügel, 1933.
Fig. 7. *Filaroides arator* (Chandler, 1931) Skriabin, 1940.
Fig. 8. *Metathelazia massinoi** (Davtian, 1933, n.emend.) Dougherty, 1943.
Fig. 9. *Parafilaroides decorus* Dougherty & Herman, 1947.

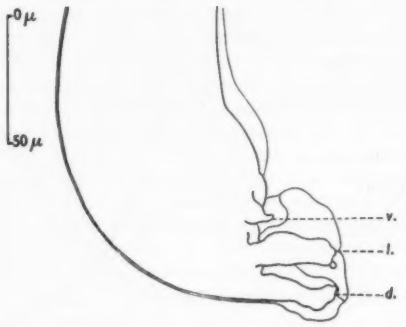
Lettering: *gub.* gubernaculum; *sp.* spicule.

The Filaroidinae have undergone specialization of the bursa and its rays by progressive loss of the bursal membrane and progressive reduction of the rays to papillae (even as far as apparent loss of the papillary rays in the genus *Parafilaroides* Dougherty, 1946). In the genera with relatively well-developed bursae (e.g. *Aelurostrongylus* Cameron, 1927) the rays are of approximately normal configuration by strongyline standards. The gubernaculum is small when present and sometimes lacking. Interestingly enough it clearly demonstrates in miniature a part of the doubled gubernacular organization that is so greatly exaggerated in the Protostrongylinae. Two horns of a tiny *capitulum* are evident, united posteriorly in an unpaired *corpus*. All six genera of the Filaroidinae are represented in the order Carnivora, and twenty-one of the twenty-nine known species. The male posterior ends of the six filaroidin genera are illustrated in Figs. 4–9.

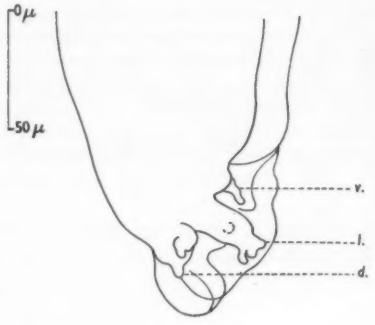
The Pseudaliinae, in contrast to the Filaroidinae, are all characterized by having radically fused and distorted rays. The ventral groups are reduced to a pair of fused rays; likewise the medial groups, which incorporate the externodorsals. There is a tendency to bursal reduction, which in *Halocercus* Baylis & Daubney, 1925, is as extreme as in *Filaroides*. The gubernaculum is simple or lacking. A unique feature of the female pseudaliins is the fact that the terminal part of the vestibulum is supplied with a

* This species was named for Prof. B. G. Massino; the trivial name is here emended from *massino* to *massinoi* for the first time.

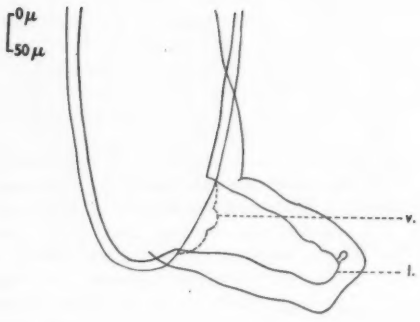
well-developed muscular sphincter. I have previously (Dougherty, 1944*b*) regarded this as a part of the vagina uterina, although this may not be true. Histological studies have not yet been done to determine the extent of the vagina vera of these forms. The term *ejector*, applied by me to this part, certain genera (e.g. *Protostrongylus*) three divisions may be recognized: an anterior *capitulum*, a middle *corpus*, and paired posterior *crura*. In other genera (e.g. *Elaphostrongylus*) no capitulum is evident. As already noted, the structure of the protostrongylin gubernaculum is merely an elaboration of that



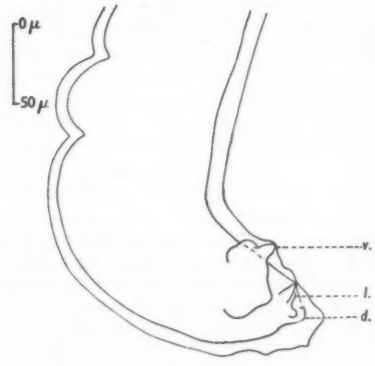
10 *Stenurus*



11 *Pharurus*



12 *Pseudalius*



13 *Halocercus*

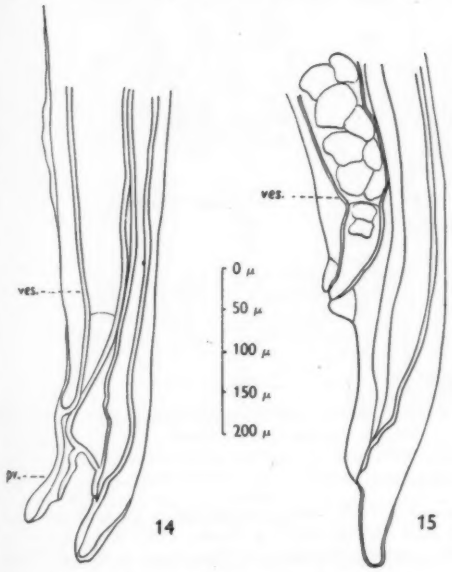
Figs. 10–13. Genera of the subfamily Pseudaliinae Railliet & Henry, 1909. Male posterior ends, lateral view. Figs. 10 and 11 from camera lucida drawings.
Fig. 10. *Stenurus vagans* (Eschricht, 1841) Dougherty, 1943 (original).
Fig. 11. *Pharurus convolutus* (Kuhn, 1829) Dougherty, 1943 (original).
Fig. 12. *Pseudalius inflexus* (Rudolphi, 1808) Schneider, 1866 (after Baylis & Daubney, 1925, fig. 2).
Fig. 13. *Halocercus kirbyi* Dougherty, 1944 (after Dougherty, 1944*b*, fig. 12).
Lettering: *d.* dorsal ray; *l.* lateral ray; *v.* ventral ray.

should be discarded. All pseudaliins are found in the suborder Odontoceti (order Cetacea). The representative bursae of the four genera of the Pseudaliinae are shown in Figs. 10–13.

The Protostrongylinae are distinguished by the remarkable elaboration of the gubernaculum. In evident in certain filaroidins. Although there is no progressive line of bursal degeneration in the subfamily, the genus *Muellerius* is characterized by striking bursal and ray reduction as illustrated in Fig. 25. A conspicuous feature of certain female protostrongylins is a cuticular prolongation of the

vagina as a sort of vulvar tube, termed the *provagina*. It is of haphazard occurrence and is not a generic feature. Protostrongylins with and without a provagina are pictured in Figs. 14 and 15 respectively. Members of all protostrongylin genera are found in hosts of the suborder Ruminantia (order Artiodactyla); of the twenty-nine species only three (all in *Protostrongylus*) occur in other hosts (order Lagomorpha). Representative male posterior ends of the ten genera of the Protostrongylinae appear in Figs. 16–25.

In all three of the subfamilies discussed there is great variability in spicular morphology from long capillary to short complex structure. Evolutionary changes in the spicules apparently can occur very rapidly, and their configuration cannot be used in the supergeneric classification of the Metastrongylidae. This fact casts doubt on the validity of subdividing the Trichostrongylidae even in part on the basis of spicular morphology, as Travassos (1937) and others have done.



Figs. 14, 15. Posterior ends of protostrongyline females, lateral view. Fig. 15 from camera lucida drawing.
Fig. 14. *P. boughtoni*, with provagina (after Goble & Dougherty, 1943, fig. 8).
Fig. 15. *Orthostrongylus macrotis* (Dikmans, 1931) Dougherty & Goble, 1946 (original).

Lettering: *pv.* provagina; *ves.* vestibulum.

The genera *Metastrongylus* and *Heterostrongylus* fit conveniently into none of the morphological groupings discussed. The former genus is not more clearly related to the protostrongylins, which characterize the same order of hosts, than to the filaroidins. I have not had the opportunity of studying, at first hand, specimens belonging to the latter genus and must therefore rely on the original figures and description given by Travassos (1925). It too fits into none of the clearly marked groups. It was incorrectly referred by me to the subfamily Skrjabingylinae in a recent paper (Dougherty, 1945*b*); it is decidedly not trichostrongylid and appears definitely metastrongylid.

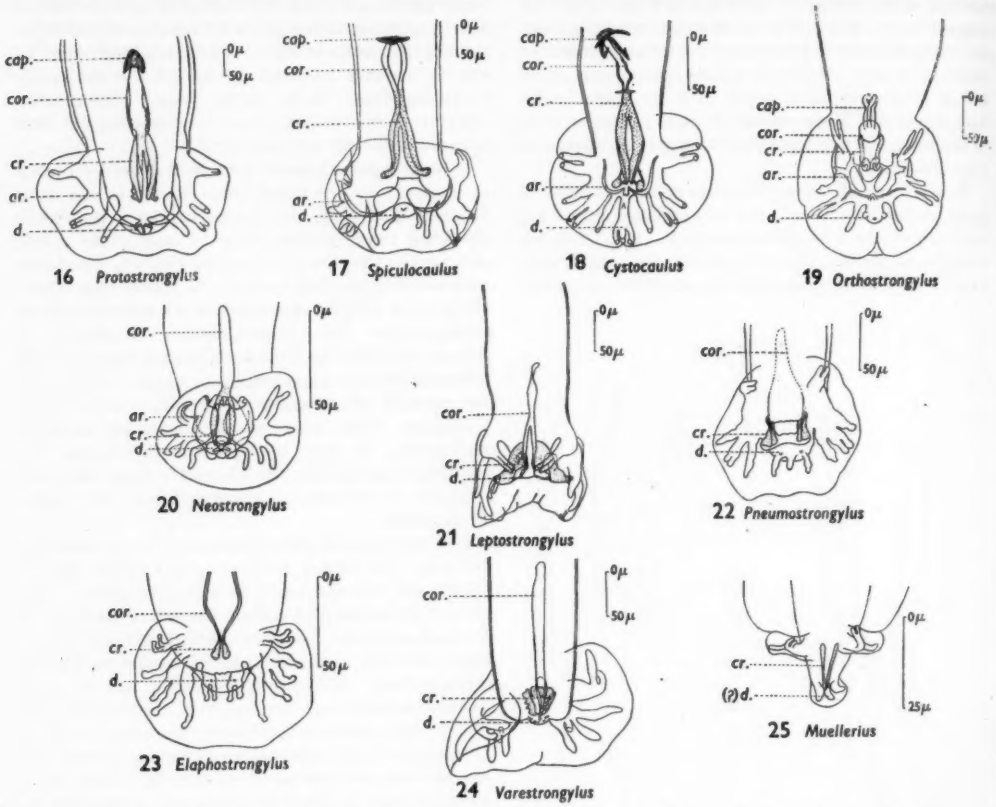
Metastrongylus has six very well-developed labia in contrast to the small labia found in the subfamilies discussed. Its bursa supports peculiarly distorted rays, unlike those of any other metastrongylid; they may be seen in Fig. 26. For these reasons I believe that it should be placed apart from the groups already discussed in a subfamily Metastrongylinae. The gubernaculum is simple or lacking; in this the Metastrongylinae resemble the Filaroidinae and Pseudaliinae. On the other hand, one species of *Metastrongylus*—*M. pudendotectus* Vostokov, 1905—possesses a somewhat atypical provagina. It may be that the provagina is a primitive metastrongylid character that has been retained by certain protostrongylins and metastrongylins.

Heterostrongylus presents some rather tantalizing features. According to Travassos's figure (No. 1) (1925) its cephalic end has a circular groove that sets off the labial region from the rest of the body as a *mouth collar* like that characteristic of the Strongylidae, but not of the Ancylostomatidae or Trichostrongylidae. Well-developed labia are obvious. Travassos speaks of three lips, but, in view of the fact that he apparently did not study the anterior end *en face*, it is probable that this is a mistake and that there are actually six lips as characteristic of the Metastrongylidae. If these features are accurately interpreted by me, then *Heterostrongylus* is a most interesting intermediate form between the Strongylidae and Metastrongylidae. In view of the fact that the host of the type and only known species—*H. heterostrongylus* Travassos, 1925—is a marsupial (the golden opossum, *Didelphis marsupialis aurita*)—its significance becomes doubly great. A decision as to its importance must, however, be made cautiously, inasmuch as its host is a South American marsupial, which could well have secondarily acquired it as a symbiote from a placentate host. In view of its suggestive primitive features, however, *Heterostrongylus* may well be a survivor of the stem metastrongylid group and have accompanied marsupial hosts from the time of their derivation from the pantotheres.

It is difficult to assign *Heterostrongylus* to a position within any of the subfamilies already established. Its gubernaculum, interestingly enough, shows, as figured by Travassos, a tendency toward the formation of a capitulum. The bursa is atypical and

somewhat like that of *Metastrongylus* in the unequal reduction of the rays. A unique feature is the wide separation of what are apparently two completely bursal membrane along with the rays or is a projection from the region of the genital cone.

I am reluctant to erect a new unigeneric subfamily



Figs. 16–25. Genera of the subfamily Protostrongylinae Kamenskiĭ, 1905. Male posterior ends, ventral view.

Fig. 16. *Protostrongylus stilesi* Dikmans, 1931 (reconstructed after Shul'ts, Orlov & Kutas, 1933, Abb. 2 and 7).

Fig. 17. *Spiculocaulus leukarti* Shul'ts, Orlov & Kutas, 1933 (reconstructed after Shul'ts, Orlov & Kutas, 1933, Abb. 4 and 8).

Fig. 18. *Cystocaulus ocreatus* (Railliet & Henry, 1907) Mikačić, 1939 (reconstructed after Shul'ts, Orlov & Kutas, 1933, Abb. 5 and 9).

Fig. 19. *Orthostrongylus macrotis* (reconstructed after Dougherty, 1945*a*, figs. 8, 9).

Fig. 20. *Neostrongylus linearis* (Marotel, 1913) Gebauer, 1932 (after Gebauer, 1932, Abb. 61).

Fig. 21. *Leptostrongylus alpenae* (Dikmans, 1935) Dougherty & Goble, 1946 (reconstructed after Dougherty, 1945*a*, figs. 4, 5).

Fig. 22. *Pneumostrongylus tenuis* Dougherty, 1945 (after Dougherty, 1945*a*, fig. 1).

Fig. 23. *Elaphostrongylus odocoilei* Hobmaier & Hobmaier, 1934 (after Hobmaier & Hobmaier, 1934, fig. 3).

Fig. 24. *Varestrongylus schulzi* (Boev & Vol'f, 1938) Dougherty & Goble, 1946 (reconstructed after Boev & Vol'f, 1938, figs. 2, 5).

Fig. 25. *Muellerius tenuispiculatus* Gebauer, 1932 (after Gebauer, 1932, Abb. 68).

Lettering: *ar.* areus; *cap.* capitulum; *cor.* corpus; *cr.* crus (or crura); *d.* dorsal ray.

divided halves of the dorsal ray and the interpolation of a ray-like unpaired process between them. It is not, however, clear from either Travassos's figure or his text whether this process is supported in the for *Heterostrongylus*, especially since I have not studied original material of the genus. In its well-developed labial structure, which is probably the most important feature phylogenetically, it is

closest to *Metastrongylus*. I therefore refer it to the subfamily Metastrongylinae. *Metastrongylus* and *Heterostrongylus* appear to be the most primitive metastrongylid genera and possibly represent somewhat specialized survivors of the stem group.

Unidentified lungworms are known to occur in North American opossums, having been recorded by Weidman (1926) and Goble & Cook (1942). The latter workers reported a microscopic slide of a section of opossum lung (*Didelphis virginiana*) showing the presence of nematodes in the bronchi. I have seen this slide. Unfortunately I have not been able to identify the sectioned organisms as to systematic position within the Nematoda.

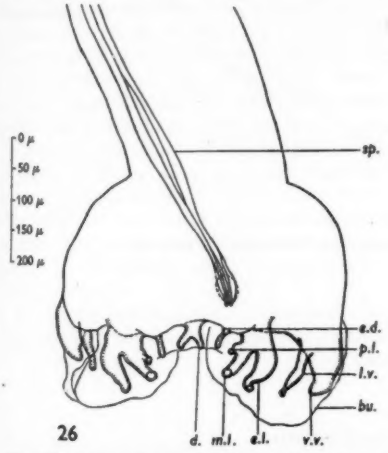


Fig. 26. *Metastrongylus pudendotectus* Vostokov, 1905. Male posterior end, dorsal view (original). *bu.* bursa; *d.* dorsal ray; *e.d.* externodorsal ray; *e.l.* externolateral ray; *l.v.* lateroventral ray; *m.l.* mediolateral ray; *p.l.* posterolateral ray; *sp.* spicule; *v.v.* ventroventral ray. From camera lucida drawing.

It is important that further lungworm material be sought in the Marsupialia, especially in Australian members of the order; such nematodes, if metastrongylid, may well divulge further basic evidence for the phylogenetic position of the Metastrongylidae within the suborder Strongylina.

My theories on the phylogenetic radiation of the Metastrongylidae are presented in Fig. 27, which also shows the evolutionary host-relationships as postulated in this paper.

V. DIAGNOSES FOR THE FAMILY METASTRONGYLIDAE AND ITS SUBFAMILIES, WITH LISTS OF COMPONENT GENERA

Diagnoses are given here for the family Metastrongylidae and its four subfamilies. Under the latter are listed the component genera.

Phylum NEMATODA

Class PHASMIDEA

Order RHABDITIDA

Suborder *STRONGYLINA*

Family METASTRONGYLIDAE Leiper, [1909]

Diagnosis. Strongylina: meiostomatous without onchia; male: bursa and rays moderately to greatly reduced, or lacking; female: vulva preanal in position, ovejectoral apparatus consisting of a long, unpaired vestibulum, short paired ejectors, and paired infundibula connecting with prodelphic uteri (ejector + infundibulum = ovejector).

Type genus: Metastrongylus Molin, 1861.

Subfamilies (4):

METASTRONGYLINAE Railliet & Henry, 1909.

Diagnosis. Metastrongylidae: six well-developed labia; male: bursa well developed but rays misshapen and irregularly reduced in size; gubernaculum simple or lacking; female: provagina present or lacking; vestibulum without terminal muscularization.

Genera (2):

(1) *Metastrongylus* Molin, 1861 (type genus) (syn. *Choerostrongylus* Gedoelst, 1923).
(2) *Heterostrongylus* Travassos, 1925.

FILAROIDINAE Skriabin, 1933.

Diagnosis. Metastrongylidae: six poorly developed labia; bursa well-developed to lacking, rays normal to papillary, or even lacking; gubernaculum sometimes showing clearly developed, though small capitulum, otherwise simple, often lacking; female: provagina absent; vestibulum without terminal muscularization; characteristic of Carnivora.

Genera (6):

(1) *Aelurostrongylus* Cameron, 1927 (syn. *Perostrongylus* Schlegel, 1934; *Pulmostrongylus* Hsü, 1935).
(2) *Angiostrongylus* Kamenskiĭ, 1905 (syn. *Haemostrongylus* Railliet & Henry, 1907; *Parastrongylus* Baylis, 1928; *Rodentocaulus* Shul'ts, Orlov & Kutas, 1933; *Pulmonema* Chen, 1935; ? *Cardionema* Yamaguti, 1941).
(3) *Gurltia* Wolffhügel, 1933.
(4) *Filaroides* v. Beneden, 1858 (syn. *Oslerus* Hall, 1921; *Filariopsis* v. Thiel, 1926; *Pseudostrongylus* Cameron, 1927).
(5) *Metathelazia* Skinker, 1931 (syn. *Osleroides* Orlov, Davtian & Liubimov in Skriabin, 1933; *Vogeloides* Orlov, Davtian & Liubimov in Skriabin, 1933; *Pneumospirura* Wu & Hu, 1938).
(6) *Parafilaroides* Dougherty, 1946.

PSEUDALIINAE Railliet & Henry, 1909.

Diagnosis. Metastrongylidae: six poorly developed labia; male: bursa moderately reduced to lacking, rays fused into five groups—paired ventral, paired medial (including the externodorsals), and unpaired dorsal—and variously reduced; gubernaculum simple or lacking; female: provagina lacking; vestibulum with terminal muscularization; characteristic of Cetacea (Odontoceti).

Genera (4):

(1) *Pseudalius* Dujardin, [1844].
(2) *Stenurus* Dujardin, [1844].
(3) *Pharurus* Leuckart, 1848 (syn. *Torynurus* Baylis & Daubney, 1925; *Otophocaenurus** Skriabin, 1942).
(4) *Halocercus* Baylis & Daubney, 1925 (syn. *Skrjabinalius* Deliamur in Skriabin, 1942).

PROTOSTRONGYLINAE Kamenskiĭ, 1905.

Diagnosis. Metastrongylidae: six poorly developed labia; male: bursa usually well developed, sometimes reduced; rays usually normal, sometimes reduced; dorsal ray sometimes mound-like and provided with several papillae; gubernaculum very much enlarged and somewhat complex, consisting often of capitulum, corpus, and crura, or of the latter two parts only; female: provagina present or lacking; vestibulum without terminal muscularization; characteristic of Artiodactyla (Ruminantia).

Genera (10):

(1) *Protostrongylus* Kamenskiĭ 1905 (syn. *Synthetocaulus* Railliet & Henry, 1907).
(2) *Spiculocaulus* Shul'ts, Orlov & Kutas, 1933.
(3) *Cystocaulus* Shul'ts, Orlov & Kutas, 1933.
(4) *Orthostrongylus* Dougherty & Goble, 1946.
(5) *Neostrongylus* Gebauer, 1932 (syn. *Neometastrongylus*† Kreis, 1944).
(6) *Leptostrongylus* Dougherty & Goble, 1946.
(7) *Pneumostrongylus* Mönnig, 1932.
(8) *Elaphostrongylus* Cameron, 1931.
(9) *Varestrongylus* Bhalerao, 1932 (syn. *Bicaulus* Shul'ts & Boev, 1940).
(10) *Muellerius* Cameron, 1927.

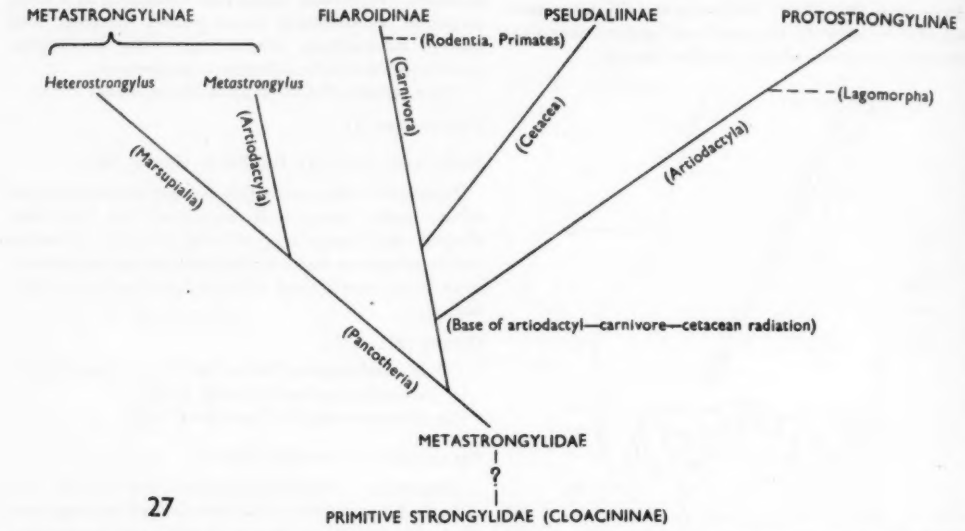


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Fig. 27. Schematic representation of the phylogeny of the family Metastrongylidae correlating symbiote and host evolution according to the author's conception. The symbiote groups are indicated in gothic capitals; the ancestral host groups (orders) in gothic lower case (in parentheses). Where members of the symbiote groups have become secondarily adapted to members of other host groups such is indicated in each case by a broken line leading to the name of the latter order or orders.

* This synonymy has not hitherto been made (see Skriabin, 1942). The type and only species of *Otophocaenurus—O. oserskoi* Skriabin, 1942, should become *Pharurus oserskaiaae* (Skriabin, 1942, emend.n.) comb.n.

† This synonymy has not hitherto been made (see Kreis, 1944). The type and only species of *Neometastrongylus—N. buechii* Kreis, 1944—is a synonym of *Neostrongylus linearis* (Marotel, 1913) Gebauer, 1932.

VI. SUMMARY

1. History of the classification of the family Metastrongylidae is briefly reviewed.

2. The probable origin of the family is discussed. It is believed that the metastrongylids arose, in common with the family Strongylidae, from a primitive group of eustomatous, cloacinin-like, gastro-intestinal strongylines that were symbiotic with an ancestral mammalian group, possibly the pantotheres. One group of these symbiotes established itself in the mammalian respiratory tract and originated the metastrongylid line.

3. The family Trichostrongylidae is regarded as of common origin with the family Ancylostomatidae from eustomatous ancestors. Thus meiostomy in the metastrongylids and trichostrongylids is convergent.

4. The Metastrongylidae consist of three main lines, each characteristic of a placentate order: the Filaroidinae in the Carnivora, the Pseudaliinae in the Cetacea, and the Protostrongylinae in the Artiodactyla. These lines probably have evolved from a common ancestor, symbiotic with a host at the base of the carnivore-cetacean-ariodactyl radiation. Certain forms have become secondarily adapted to symbiosis with members of other placentate orders.

5. Morphological and biological evidence is given for each of the foregoing phylogenetic conclusions.

6. *Metastrongylus* Molin, 1861, is regarded as a relict genus. With it, in a subfamily Metastrongylinae, is placed the genus *Heterostrongylus* Travassos, 1925.

7. *Heterostrongylus* is represented by a single known species, *H. heterostrongylus* Travassos, 1925, from an opossum (order Marsupialia). Morphologically it is primitive with cloacinin-like cephalic features. In view of the phylogenetic status of its host it may well represent an ancestral type that has evolved along with the Marsupialia from a common ancestry with the other metastrongylids, probably in the panthotheres. As yet unknown lungworms of marsupials may shed light on this interesting point.

8. Diagnoses are given for the family Metastrongylidae and its subfamilies; the twenty-two component genera are listed under the latter. New synonymies are made: *Neometastrongylus* Kreis, 1944, a synonym of *Neostrongylus* Gebauer, 1932; *Neometastrongylus buechii* Kreis, 1944, of *Neostrongylus linearis* (Marotel, 1913) Gebauer, 1932; and *Otophocaenurus* Skriabin, 1942, of *Pharurus* Leuckart, 1848. *Otophocaenurus oserskoi* Skriabin, 1942, becomes *Pharurus oserskaiaae* (Skriabin, 1942, n.emend.) n.comb.; *Metathelazia massino* becomes *M. massinoi* (Davtian in Skriabin, 1933, n.emend.) Dougherty, 1943.

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* Since the publication of an earlier paper in which this reference was cited (Dougherty & Goble, 1946, footnote 4), the latter has been obtained on microfilm through the courtesy of Dr W. A. Pool, Director of the Imperial Bureau of Animal Health, Weybridge, Surrey, England.

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THE SCUTUM OF THE TICK, *IXODES RICINUS* L.

By D. R. ARTHUR, M.Sc., *Department of Zoology, King's College, University of London*

(With 9 Figures in the Text)

It is a common observation that in individual *Ixodes ricinus* L. ticks, size variations occur; such variation is reflected in the records of early workers (Wheler, 1899; Neumann, 1899: quoted by Nuttall, Warburton, Cooper & Robinson, 1911). The present paper attempts to examine the true specific importance of size variation by basing the study on measurements of the relatively inelastic scuta after the principle of using head capsule measurements for insectan larval instars.

Average dimensions of the scuta of the male and female only of this species have been given by Nuttall *et al.* (1911), but no indication is given as to whether they were determined while the scuta were in position on the dorsum or after removal. The measurement of the scutum of *I. ricinus in situ* is fraught with risks due to the lack of delineation afforded by the marginal areas relative to the extra-scutal cuticle. This feature precluded the adoption of the method used so successfully by Cunliffe (1913) in the measurement of the scuta of *Rhipicephalus pulchellus* Gerstäcker.

In the present work, therefore, the determinations of size were undertaken after the removal of the scuta and mounting on a micro-slide. This method is the nearest approach to accuracy in such pigmented ticks, despite the fact that determinations are being made of a slightly curved surface depressed under a cover glass.

The structures were removed from all stages of the tick by means of an extra-fine pair of lancet needles under a binocular microscope, great care being taken to avoid damage to the marginal areas. Where, on occasions, the scutum was not removed with ease from the attached cuticle, staining with an aqueous 1% acid fuchsin stain for 3 min. proved exceptionally valuable. The extra-scutal cuticle took up the stain more rapidly than the scutum, thus sharply differentiating the latter from the background.

The length (L) of the scutum of all phases was measured from the midpoint of a line connecting the apices of the scapulae to the posterior border (Fig. 1). The line connecting the scapulae was also measured (B_1) and the width (B_2), being the distance between the two most lateral points. All measurements were made with a micrometer eyepiece and the aforementioned method has also been used to determine the scutal growth rate from larva to adult.

Variation in tick scuta resolves itself into two themes, (*a*) intraphase variation, and (*b*) interphase variation. In the present paper particular attention has been directed to variations in size and form, colour and chaetotaxy.

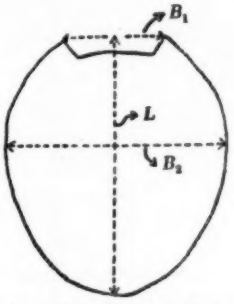

Fig. 1. The scutum of *Ixodes ricinus* L. to show methods of measurement employed in the text.

VARIATION IN SIZE AND FORM

According to Nuttall *et al.* (1911) the larva of *Ixodes ricinus* has 'the essential characters of the nymph', a statement which is ambiguous in many respects. The material employed in the present survey had its origin in larvae derived from a number of egg clusters obtained from both unimodal and bimodal belts of seasonal activity (Edwards & Arthur, 1947).

The length (L) of the larval scuta measured varied from 0·30 to 0·34 mm., with a mean of 0·328 mm., S.D. = 0·09, the breadth (B_2) from 0·35 to 0·39 mm., with a mean of 0·375 mm., S.D. = 0·10 (Fig. 2). The range in both cases was 0·04 mm. A significant feature is that the width (B_2) exceeds the length with the result that L/B_2 is always less than unity (Fig. 5). Whilst the figures quoted indicate extreme ranges it is interesting to note that 90·6% of the length measurements lie between 0·32 and 0·34 mm., while for breadth dimensions 80·1% fall within the limits of 0·37 and 0·39 mm.

The length and breadth of the scutum of nymphs range from 0·56 to 0·70 mm. and 0·515 to 0·67 mm. respectively, with corresponding means of 0·62 mm. (S.D. = 0·03) and 0·59 mm. (S.D. = 0·033). The length

is almost always greater than the width (B_2) and L/B_2 fluctuates between 0·97 and 1·13 (Fig. 5) as shown below (based on 404 measurements).

Frequency (%)	Ratio L/B_2
2·62	0·97
4·70	0·98
3·71	0·99
88·97	> 1·00

Generally speaking the larger specimens have a somewhat truncated appearance which contrasts with the more rounded form of the 'dwarf'. A comparison of Figs. 2 and 3 will show that in the nymphal scuta there is a far greater vertical and horizontal scatter about each point than is true for the larvae.

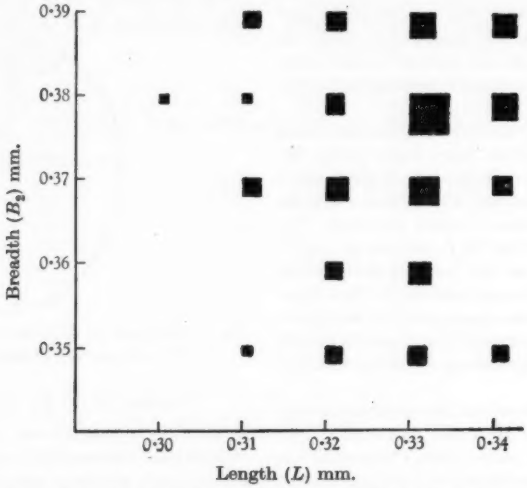

Fig. 2. Correlation diagram of the length and breadth of *Ixodes ricinus* larvae. The area of each square is proportional to the number of samples occurring at each point.

In the light of the dual measurements of females presented by Nuttall *et al.* (1911) it would be expected on measuring a large number of specimens that two modes would occur when length-breadth ratios are graphed, and that these modes would be 1·13 or 1·33 according as to whether length × breadth approximated to 1·3 × 1·15 mm. or to 1·4 × 1·05 mm. The curve of the L-B_2 ratios (Fig. 5) for females, based on 898 determinations, shows no evidence of two such peaks; nor is there any evidence in my measurements of scuta from sheep, cattle, goats, horses and dogs of different frequency modes according to the size of the host. Such a bimodal frequency is, however, recorded by Mickel (1924) for a species of *Dasymutilla* according as to whether it parasitizes the lesser-sized *Microbembix* or the larger *Bembix* wasp. The results, however, do suggest that this character only obeys the laws of chance, that is to say, the distribution of size about the average is governed by no special factor and the statistical material is homogeneous.

For the comparison of scutal increase in relation to phase changes, the technique adopted involved breeding out larvae from a single egg cluster and the determination of the mean dimensions of the scuta. One hundred larval scuta were measured.

Subsequently several hundred larvae from the original cluster were placed on hedgehogs in a special cage overlying a dish of water. When completely engorged the larvae dropped off the host, through the floor grating and into the water, whence they were collected, transferred to small cages contained in tubes (Langeron, quoted by MacLeod, 1932) and allowed to metamorphose to nymphs. A large number of metamorphosed nymphs likewise fed and developed to the adult stage. The results obtained are presented in Table 1.

Table 1. *Dimensions of the scuta of various phases of* Ixodes ricinus *L.*

Phase	No. measured	Mean scutal dimensions (mm.) Length	Breadth (B_2)	Breadth (B_1)
Larvae	100	0·33	0·38	0·14
Nymphs	100	0·62	0·59	0·24
Females	79	1·38	1·25	0·57
Males	21	1·78	1·15	0·54

From the data it is seen that the increase of the adult scutal dimensions over those of the nymph, and

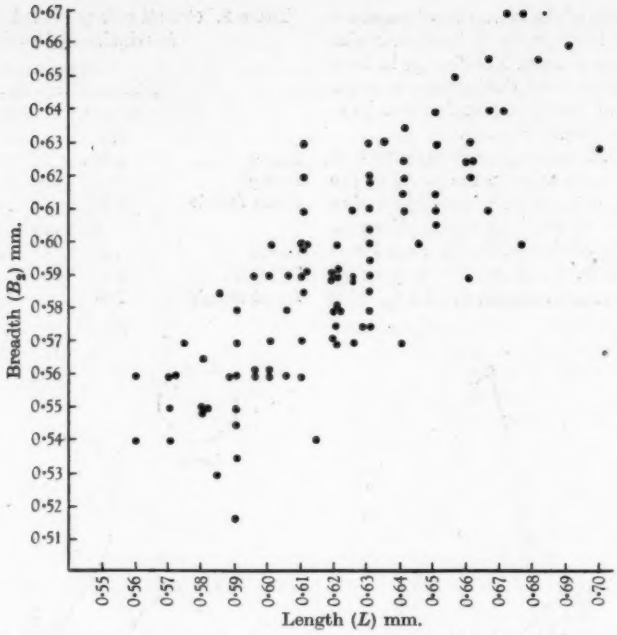

Fig. 3. Correlation diagram of the length and breadth of *Ixodes ricinus* nymphs.

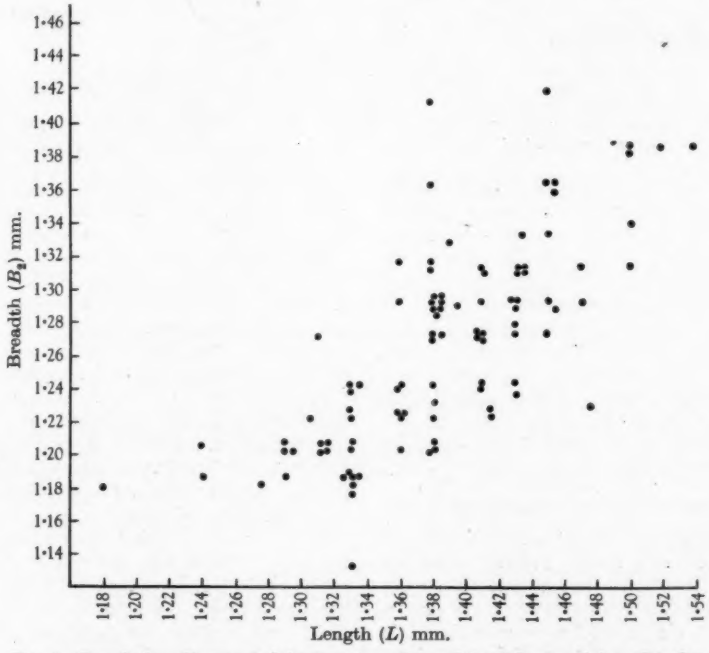

Fig. 4. Correlation diagram of the length and breadth of *Ixodes ricinus* females.

of the nymph over those of the larvae is not constant for all measurements; in other words, increase in size brings with it a change of form. The change in form of the adult male scutum from the immature stages is visually obvious, but the corresponding change in the female is not so apparent (Fig. 6).

Considering first of all the length of the scutum, it will be seen that the increase from the larval to the nymphal scutum is 87·8 %, while the corresponding increase from nymph to adult female is 122·6 %. From the larva to the adult female the total mean increase in length is 318·2 %. From the larva to the nymph the scutal breadth increase is 55·3 %. This

Table 2. *Growth rate of the scuta of* Ixodes ricinus *in relation to Dyar's Law*

	Observed values (mm.)		
	Length (L)	Breadth (B_2)	Breadth (B_1)
Larva	0·33	0·38	0·14
Nymph	0·62	0·59	0·24
Adult female	1·38	1·25	0·57
	Calculated values (mm.)		
Larva	—	—	—
Nymph	0·68	0·71	0·24
Adult female	1·27	1·06	0·41

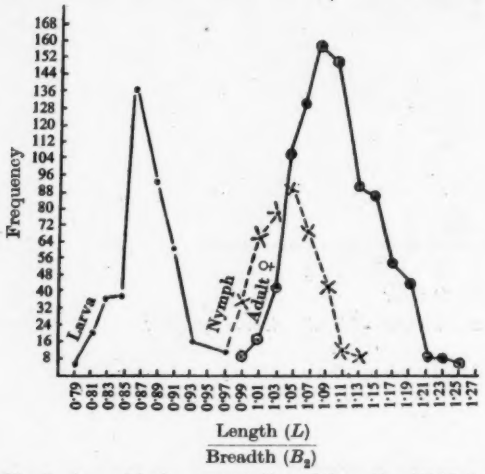

Fig. 5. A graphical representation of the ratio of L/B_2 for larvae, nymphs and adult females of *Ixodes ricinus*.

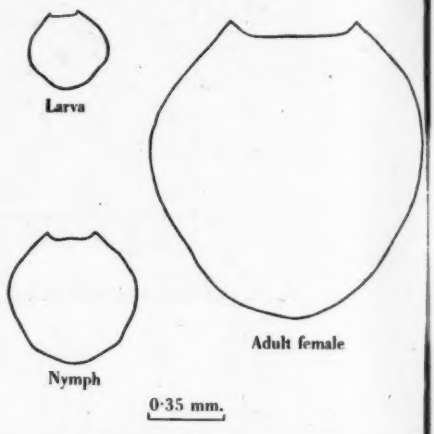

Fig. 6. Shape and relative sizes of the scuta of larvae, nymphs and adults of *Ixodes ricinus*.

unequal dimensional increase of length and breadth is sufficient to alter the ratio of L/B_2 from a quantity less than unity (in the larvae) to a value exceeding this (in the nymphs). The breadth increase of the female over the nymph is 113·6 %, giving an overall increase from larva to adult female of 229 %. In other words, the total mean increase in length is 1·39 times that of the breadth. The nymphal scutum shows an average surface area increase of 206 % over that of the larva, while the corresponding figure when comparing nymph and adult is 358 %.

The growth of the scutum of *Ixodes ricinus* shows no conformity with Dyar's Law, as seen in Table 2.

VARIATION IN CHAETOTAXY

There are two types of setae on the scutum, viz. the primary and secondary setae. Primary setae are those which are present on the larval scutum, while the additional setae which occur after the first or succeeding moult are referred to as secondary. In *Ixodes ricinus* and other Ixodid ticks additional setae are gained at succeeding moults so that the chaetotaxy of the scutum at the mature stage is distinctly different from that of the larval scutum.

The primary setae are remarkably constant in number and position in the scutum of the larva of *I. ricinus* and may provide important diagnostic features for the separation of species, particularly in the larval instar. Twenty-three primary setae are recognized: of these ten are long setae (Fig. 7*a*) which may be referred to as tactile setae, four are of the type depicted in Fig. 7*b* (henceforth referred to as 'α type') and nine are of the type indicated in Fig. 7*c* (henceforth referred to as 'β type'). The tactile setae present on the scutum are shorter than those present on the extra-scutal cuticle.

For purposes of scutal chaetotaxy, the structure has been divided into three fields (Fig. 7*d*). The median field (M) lies between the cervical grooves; the lateral field (L) between the cervical grooves and the lateral carinae; the marginal field (C) located laterally to the lateral carinae.

One of the principal characteristics of the larval scutum is its relative scarcity of the tactile and 'β' setae when compared with both nymphal and adult forms (Fig. 8*a*). In *M* there are four tactile setae (T_1–T_4), one at each angle near the anterior end of the cervical groove and a pair midway along this natural division. Set obliquely to T_3 and T_4 on each side are single elements of β type (B_2 and B_3) and of α type (P_1 and P_2). In the midline, and posterior

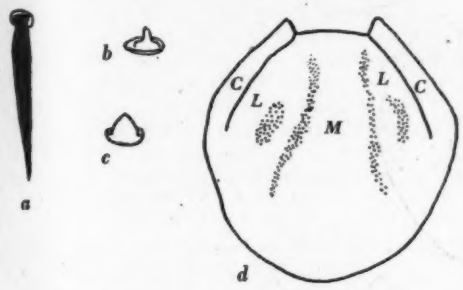

Fig. 7. Setal types found on the scutum of *Ixodes ricinus* (*a*, *b*, *c*) and the zones used in the chaetotaxis study (*d*).

symmetrical or asymmetrical. The number of tactile setae increases in the stages succeeding the first moult and in the nymph they form a distinct transverse row (Fig. 8*b*). This increased setal density becomes more accentuated in the adult female, though the linear arrangement becomes more disorganized. Tactile setae in the nymphal stage number typically 28. The β setae are, however, less constant in these respects and may number 40–60 in the majority of cases.

VARIATION IN COLOUR

The colours in preserved Ixodid specimens are subject to modification and the lighter may become as dark as those which are of an intense brown in the living state. In the present work scutal colour variation has been determined in living material. The differences of colour are the results of variation in pigmentation intensity and extent. Basically the scuta are brown in colour, varying from yellow to a blackish brown. In the females and nymphs there is a significant lack of uniform coloration, some parts of the structure being more intensely pigmented than others. Only one instance of a scutum with uniform coloration was found

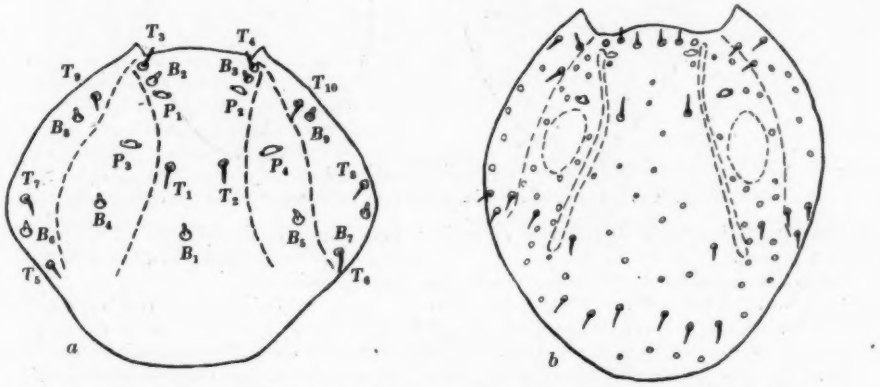

Fig. 8. *a*, the chaetotaxy of the larva; *b*, the chaetotaxy of the nymph of *Ixodes ricinus*.

to T_1 and T_2, is a single β seta (B_1). In the lateral field are two microscopic setae: the more anterior, at about a third of the length, is of the α type (P_3 and P_4) with a β type (B_4 and B_5) located posteriorly. The marginal group (*C*) includes three tactile setae (T_{5-10}) on each side, arranged marginally, while intermediate in position are the β setae (B_{6-9}).

As in the larva, the α setae of the nymph number four and they occupy the same relative position in both phases. In the adult they retain the same approximate disposition, but instances have been noted where duplication occurs, this being either

during the examination of 2250 females and 1780 nymphs. In the majority of nymphal and female structures the anterior angle, marginal to the lateral groove and including the scapulae, is generally darker than the remainder, with the cervical grooves invariably lighter. In extreme specimens the zone, delineated above, is blackish brown, but where the ground colour tends to be yellow the degree of pigmentation of the anterior marginal borders and scapulae is correspondingly less.

The most prevalent conditions of colour differentiation in females are presented in Fig. 9, but it should be realized that these differences are of degree and

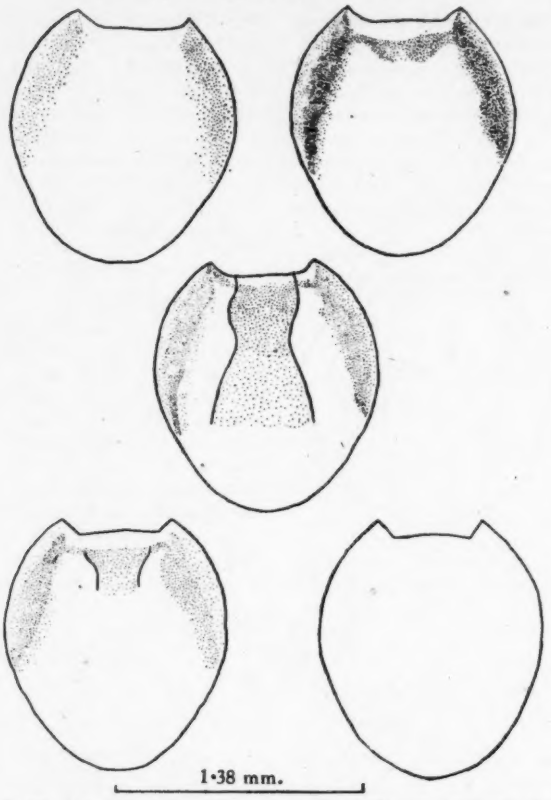

Fig. 9. The colour variation in the scuta of the female of *Ixodes ricinus*.

a complete gradation between the two extremes has been observed.

In the adult female the cervical grooves are superficial, barely reaching the posterior border, while the lateral grooves are fairly distinct and extend about half the length of the scutum. In the larvae and nymphs the cervical grooves are more distinct, being paler than the rest of the scutum, whilst intermediate in position between the cervical and lateral grooves there is a distinct oval area of pale colour. This latter area is less apparent in the adult female. Both the cervical grooves and the oval area show a more pronounced reticulate pattern than the rest of the scutum. In contrast the larvae, apart from the lighter areas of the cervical grooves and the afore-mentioned oval area, show evenness of pigmentation.

Collections of ticks in Wales have been undertaken in rush, bracken, heather, sphagnum moss and rough grasses and from their hosts, and it was of interest to find similar ranges within different habitats, not far removed from one another geographically.

The writer is indebted to Mr T. W. Tyssul-Jones, M.Sc., for his assistance in checking the measurements, to Mr L. F. Cowley, Dr B. M. Hobby and Dr J. MacLeod for their ready help at all times and for reading the original script.

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REACTIONS OF *CYPHOMANDRA BETACEA* TO STRAINS OF POTATO VIRUS *X*

By R. E. F. MATTHEWS, *Plant Virus Research Unit, Molteno Institute, University of Cambridge*

Experimental evidence is given which shows that severe strains of Potato virus *X* may be selected out from a pre-existing mixture of mild and severe strains, on inoculation to *Cyphomandra betacea*.

The results are discussed in relation to similar findings by other workers. It is suggested that, in general, such changes are more likely to occur by selection from a pre-existing mixture of strains than by a 'specific' or 'directed' mutation caused by the presence of the virus in the particular host.

INTRODUCTION

During the course of work carried out in 1941 at the Plant Diseases Division, Auckland, N.Z., on a mixture of *X* strains in Red Dakota potatoes, it was found that among the twenty solanaceous species tested in host range studies, *Cyphomandra betacea* gave an anomalous reaction.

Inoculation from Dakota potatoes to *Nicotiana tabacum* produced numerous chlorotic local lesions and a very few ringspot local lesions, followed by systemic green veinbanding and irregular mottling, with scattered necrotic spots. These symptoms indicated a mixture of strains, predominantly mild, with a small amount of severe strain. Back inoculation to tobacco from eighteen other solanaceous species, inoculated from Dakota potato, produced essentially similar symptoms.

Passage of the mixture from Dakota potatoes through *Cyphomandra* to *Nicotiana tabacum* produced a markedly different result, consisting of numerous ringspot, and no chlorotic, local lesions, followed by systemic ringspot type symptoms. As this symptom picture persisted in successive transfers through various solanaceous hosts, it was apparent that passage through *Cyphomandra* had caused a change in the virus strain or strains present, and that the change was towards an increase in severity, as judged by symptoms on tobacco.

Further work has been carried out to gain information on the nature of this change. For convenience, the terms 'mild' and 'severe' will be used to describe the strains used in the present work, mild strains being those that usually give only mild mottle symptoms on tobacco and severe strains those that give necrotic etched and ringspot symptoms under similar conditions in the same host plant. By using this definition it is not intended to suggest either that tobacco is a particularly important host for classifying *X* strains, or that all *X* strains fall into two groups as judged by symptoms on tobacco.

I. PASSAGE OF *X* STRAINS FROM VARIOUS SOURCES THROUGH *CYPHOMANDRA*

Each test was made by inoculation from the source to two or three *Cyphomandra* plants and two or three White Burley tobacco plants. When symptoms had

Source of virus	No. of tests	Result of passage
(*a*) Mild strains—sources with no evidence of strain mixture:		
(1) Virus *B* from Duke of York	3	All no apparent change
(2) Strain from tomato	4	All no apparent change
(*b*) Severe strain—source with no evidence of strain mixture:		
(3) From Arran Peak potato	1	No change
(*c*) Sources known to be mixtures of predominantly mild with a little severe strain:		
(4) From a potato from Palestine	4	Increase in severity in all four tests
(5) Potato—variety unknown	4	Increase in severity in three tests. No change in one test
(6) Arran Victory potato	1	No apparent change
(7) Potato, variety unknown	1	Increase in severity
(8) Red Dakota potato	4	Change to apparently pure severe strain
(9) Red Dakota potato	2	Marked increase in severity
(10) Auckland Short Top potato	1	Marked and apparently fairly complete change to severe strain
(11) Auckland Short Top potato	1	
(12) Salaman's X^L ex President potato	3	Increase in severity in two tests. No apparent change in one test
(13) Clinch's severe *X* ex recovered Arran Victory plant	1	Marked increase in severity

appeared inoculations were made from the *Cyphomandra* and the control tobacco plants to fresh tobaccos and any visible change in the strains present was noted.

Thus in seven tests with two strain sources believed not to contain any severe strain no change occurred. In nineteen out of twenty-two tests with known mixtures of mild and severe strains there was an increase in severity.

The amount of increase in severity was not the same in every test. In three tests there was no apparent change. In most tests there was a marked increase in severity, but some mild strain still remained. In some tests the change to severe strain type was apparently complete.

These results strongly suggest that passage through *Cyphomandra* causes an increase in severity by selection of more severe strain types from a pre-existing mixture, rather than by causing mutation of the strains introduced.

II. STAGE AFTER INOCULATION AT WHICH THE CHANGE TAKES PLACE

(*a*) *Isolations from inoculated leaves*

Inoculation of a mixture of a mild strain with a little severe strain to *Cyphomandra* usually produces a few scattered chlorotic, or black necrotic irregular local lesions. From these lesions the severe strain can be isolated in greatly increased concentration; while from the symptomless areas between these local lesions the mild mixture in essentially its original form may be isolated. These isolations have been repeated for two different sources of *X* strain mixtures.

(*b*) *Isolations from non-inoculated leaves*

In two tests inoculations to tobacco from non-inoculated leaves of *Cyphomandra* were made on successive days after inoculation with a mixture of strains. The first virus to be detected in the systemically infected leaves was of the severe necrotic type.

These experiments show that on inoculation with a mixture of strains, both may enter and multiply in the inoculated leaf, but that virus of the severe necrotic type may be isolated at an early stage from the non-inoculated leaves. Thus it is apparent that the change takes place while the virus is moving into and multiplying systemically in the plant.

III. THE CONCENTRATION OF *X* STRAINS IN *CYPHOMANDRA* AND TOBACCO

Sap was extracted in the usual way from the plants to be tested. The concentration of virus in each sample was estimated serologically and by local lesion counts.

Local lesion counts. Ten-fold serial dilutions of each pair of samples to be compared were inoculated to opposite half leaves of White Burley tobacco plants. Local lesion counts were made about 8 days after inoculation.

Serological estimation. The two rabbit antisera used were prepared by injection of partially purified virus preparations (strain mixtures from two different sources). Relative virus concentrations in two or more samples were determined either by optimal proportions (two-fold serial dilutions of antiserum) or by the virus end-point.

(*a*) *Concentration of severe strain in tobacco and* Cyphomandra

Material. A tobacco plant and a *Cyphomandra* plant inoculated at the same time from Red Dakota potato and kept under the same conditions.

Results of local lesion counts (Table 1) show that there is about 300 times as much local lesion forming (severe strain) virus in *Cyphomandra* as in tobacco.

Table 1. *No. of necrotic local lesions (mean of eight half leaves)*

Sap from tobacco		Sap from *Cyphomandra*	
Dilution of sap	No. of local lesions	Dilution of sap	No. of local lesions
10^{-1}	41·4	10^{-3}	143·0
10^{-2}	8·6	10^{-4}	23·4
10^{-3}	1·3	10^{-5}	4·3

(*b*) *Concentration of severe strain in tobacco after passage through* Cyphomandra

Material. Two tobacco plants—one inoculated with original mixture from Red Dakota potatoes, and the other inoculated from *Cyphomandra* and showing severe (ringspot type) symptoms.

Estimated serologically (by virus end-point) there was four times as much virus in the tobacco with the original culture as in the tobacco with the severe type from *Cyphomandra*. This estimate is consistent with the fact that in tobacco plants systemically infected with severe strains of potato virus *X* there may be large areas of leaf, between the systemic rings, from which no virus can be isolated.

Table 2. *No. of necrotic local lesions (mean of eight half leaves)*

Tobacco (ex Dakota)		Tobacco (ex *Cyphomandra*)	
Dilution of sap	No. of local lesions	Dilution of sap	No. of local lesions
10^{0}	29·1	10^{-3}	185·1
10^{-1}	45·75	10^{-3}	52·75
10^{-2}	22·5	10^{-4}	5·25
10^{-3}	4·4	10^{-5}	2·1

Results of local lesion counts (Table 2) show that there was about fifty times as much ringspot-forming strain in the tobacco plant inoculated directly from *Cyphomandra* as in that inoculated directly from potato (even though there was only one-quarter as much virus present—taking the serological figure as an estimate of the total virus present in each case).

(c) *The concentration of different strains in* Cyphomandra *and tobacco*

Material. Three plants were used, grown under the same conditions and infected as follows:

(1) A tobacco plant infected with a mixture of *X* strains (mainly mild) from Dakota potato.

(2) A plant of *Cyphomandra* inoculated from the Dakota source and containing the ringspot type of strain only (as judged by inoculation to tobacco).

(3) A tobacco plant containing the ringspot strain from *Cyphomandra*.

All three plants had been systemically infected for some weeks before the tests were made.

Table 3 gives the ratios of concentrations in sap from the three plants determined by β optimal proportions, using two different antisera. The sample with the lowest concentration is given the value of unity.

Table 3. *Relative virus concentrations determined serologically*

Source	1st antiserum	2nd antiserum
(1) Tobacco with (mainly) mild strain ex Dakota	16	8
(2) *Cyphomandra* inoculated ex Dakota	2	2
(3) Tobacco with ringspot strain from Dakota	1	1

Table 4 gives the results of local lesion counts, each figure in the table being the mean number of necrotic local lesions from ten to fourteen half leaves.

Table 4. *No. of local lesions (means of ten to fourteen half leaves)*

Dilution of sap	Plant no. (1)	(2)	(3)
10^{0}	4·3	170·5	—
10^{-1}	23·9	144	122
10^{-2}	1·5	95·1	35
10^{-3}	0	49	15·1
10^{-4}	—	14	2·5
10^{-5}	—	12·6	1·2

For the comparison of concentration of the severe strains in tobacco (3) and *Cyphomandra* (2) the two estimations give essentially the same result within the limits of the methods: i.e. the ringspot strain exists in two to three times the concentration in *Cyphomandra* compared with tobacco.

Estimated serologically there is about four to eight times as much virus in (1) as in (2) (Table 3). Estimation of severe strain only by local lesion counts (Table 4) shows fifty to one hundred times as much severe strain in *Cyphomandra* as in the original strain mixture from Dakota in tobacco.

Comparison of (1) and (3) (Table 4) shows that the concentration of severe strain in tobacco is increased several hundred times by passage through *Cyphomandra*.

It has not been possible, so far, to isolate the mild strains from mixtures in pure form, even by single lesion culture from high dilutions.

Therefore it has not been possible to make a direct comparison of concentration or rate of movement in *Cyphomandra* between mild and severe strains from a mixture which is changed by passage through *Cyphomandra*.

However, the results available show that:

(i) Passage of the mixture from Dakota through *Cyphomandra* causes an increase of the order of 100-fold in the amount of severe strain in tobacco.

(ii) The severe strain alone is two to three times as concentrated in *Cyphomandra* as in tobacco.

(iii) The mild strain (with a little severe strain present) is four to eight times as concentrated in tobacco as the severe strain alone. Therefore, judged by concentration reached, *Cyphomandra* is a better host for the severe strain than tobacco. Tobacco is a better host for the mild strain than for the severe strain.

DISCUSSION

Until much more is known of the facts of virus multiplication in the plant, no real explanation can be given as to why *Cyphomandra* is a better medium for the multiplication of severe strains.

The phenomenon must be due to differences in the strains of virus—differences which, in the other hosts tested, are given no chance of being made manifest, but which in *Cyphomandra* result in the dominance of severe strains over mild ones.

Cyphomandra must differ from the other Solanaceae tested in that, in its tissues, these particular strain differences can show up as they do.

When more facts are known of the differences between the virus strains, and when methods are available for a closer study of the physiology of the hosts, it may be that a comparative study of *Cyphomandra* and some other Solanaceae will throw some light on the conditions and requirements for virus synthesis.

The experimental evidence presented indicates that the phenomenon is one of selection of strains already present. Even if it seemed likely that the

phenomenon was due to mutation this would be extremely difficult to prove experimentally. If it was suggested that on the introduction of a mild strain into *Cyphomandra* this host caused a mutation to a severe strain, it would seem impossible to distinguish between mutations occurring spontaneously and favoured for survival in *Cyphomandra* (but *not* in tobacco) and mutations caused by the presence of the mild strain in this particular host.

There have been several records of somewhat similar phenomena for this and other viruses.

Carsner (1925) showed that the virus causing sugar-beet curly top could be altered by transmission to *Chenopodium murale* because when returned to beet from this plant it produced only mild symptoms.

Lackey (1932) showed that the change was reversible and that the virus could be returned to its original condition by passage through *Stellaria media*.

According to Salaman (1938) a strain of potato virus *X* causing ringspot symptoms, when inoculated into seedling beet, produced small necrotic local rings only. When the virus from the local lesions was returned to tobacco only faint mottle developed instead of ringspot symptoms.

Clinch (1944) describes an interesting case in which a severe strain of potato virus *X*, found arising naturally in seed potato stocks, underwent a striking decrease in virulence over a period of several seasons in all varieties tested. She tentatively favoured the view that the change was due to a mutation of the severe strain to a mild form after the initial inoculation. Speculating on the origin of the severe strain, she suggested the possibility that there may be other host species in which mild strains revert to severe types. Clinch's 'severe *X*' from a 'recovered' Arran Victory plant has been passed through *Cyphomandra* with a resulting marked increase in severity of symptoms in tobacco.

More recently, Johnson (1947) has shown that passage of ordinary severe type tobacco mosaic through Sea Holly results in mild symptoms only on tobacco. He has shown that severe strains move more slowly in Sea Holly and thus this plant acts as a 'filter' host separating the mild strain without any true mutation in Sea Holly.

In view of this work with tobacco mosaic virus, together with the present results for potato virus *X*, it seems probable that, in other cases where passage through a particular host has led to a change in the symptom picture, the change is due to selection of strains rather than to a mutation induced by the host.

My best thanks are due to Dr Phyllis Clinch for material containing her 'severe *X*' strains.

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THE UPTAKE OF RADIOACTIVE PHOSPHORUS FROM HOST TISSUES AND FLUIDS BY NEMATODE PARASITES

By W. P. ROGERS and MARIAN LAZARUS

Australian Council for Scientific and Industrial Research, McMaster Animal Health Laboratory, Sydney, N.S.W.

(With 5 Figures in the Text)

INTRODUCTION

The occurrence of phosphorus in *Ascaris lumbricoides* of the pig and *Parascaris equorum* has been determined by Flury (1912) and Fauré-Fremiet (1913). Flury recorded the phosphorus content of whole *Ascaris lumbricoides* as 430 mg./100 g. of dry tissue and Fauré-Fremiet showed that 0·4–0·6 % of the dry weight of the ovaries was attributable to calcium phosphate of the 'boules hyalines'.

Many features of the phosphorus metabolism of parasitic nematodes have been recorded. Thus, the degradation of phosphorus compounds in incubated brei was demonstrated by Schulte (1917) and Fischer (1924). The high phosphorus turnover associated with the egg production of *Haemonchus contortus* has been determined by Martin & Clunies Ross (1934).

The importance of carbohydrate metabolism in the adult nematode is suggested by the predominance of glycogen among the reserve substances of the organism. The glycogen content of *Ascaris lumbricoides* may reach 5·7 % of the living worm (v. Brand, 1937). Considerable amounts of glycogen have also been found in *Parascaris equorum* (Toryu, 1935) and *Ascaridia galli* (Reid, 1945). The demonstration of similar reserve substances in smaller forms has been carried out by Giovannola (1936) and Rogers (1948). Glycogen in nematode parasites is metabolized largely through phosphorylative routes (Rogers, unpublished), and the demand for phosphorus by these organisms must be great. The present publication gives an account of the rate and route of uptake of inorganic phosphate by *A. galli*, *Nippostrongylus muris* and *Ascaris lumbricoides* (pig strain). The distribution of total phosphorus in the tissues of *A. lumbricoides* is also given.

MATERIAL

Nippostrongylus muris was obtained from experimentally infected rats as described previously (Rogers, 1948). *Ascaridia galli* was maintained in young chickens, the adult worms being used some 6 weeks after infecting the host birds with eggs. *In vitro* experiments with this parasite were commenced within 30 min. of the death of the host. *Ascaris lumbricoides* (pig strain) was brought to the laboratory in vacuum flasks containing the medium described by Baldwin (1943). Experiments on the route of uptake of radioactive phosphorus were commenced within 3 hr. of the death of the host.

Samples for the determination of total phosphorus were taken from large, reddish female *A. lumbricoides*. Perienteric fluid was obtained by drainage through a small incision made in the body wall of each worm. Care was taken to avoid undue damage to the muscular or reproductive tissues. Samples of the muscular tissue were obtained by opening the parasites along one of the lateral lines, removing the intestine and reproductive organs, and scraping the muscles free from the cuticle. The material was then quickly washed in ice-cold distilled water and excess water removed with filter-paper. Intestines and reproductive organs were also washed and dried, but, in addition, the lumen of each intestine was washed with cold water injected with a syringe.

METHODS

Radioactive phosphorus (P^{32}) was used as disodium hydrogen phosphate. Samples for counting were obtained by partial wet ashing of tissues with nitric, hydrochloric and perchloric acids. After a large proportion of the acids had been driven off by careful heating, the solutions were diluted and suitable samples measured into standard counting dishes and dried at 104° C. The P^{32} was determined be means of a Geiger-Müller counter and scaling unit. The very small parasites, *Nippostrongylus muris*, were not ashed for counting, but were collected and dried on counting dishes. The maximum amounts used did not reach 5 mg./sq.cm. of counting area. In all cases, materials used for counting were exposed to the Geiger tube in very thin layers to avoid appreciable self-absorption within the sample. Counting times and samples were selected to give, as a rule,

a maximum error of 5% in the determination of radioactivity, though in certain instances, as when P^{32} in very small amounts of *N. muris* was determined, the maximum error was somewhat greater.

The wet weight of *N. muris* was found by weighing known numbers of parasites (usually about 800, each lot taken from the one host) which had been washed in saline and the excess fluid removed with filter-paper. In three determinations it was found that the weight of the mixed female and male worms approximated to 40 mg./1000. From this figure the weights of *N. muris* used in the samples prepared for counting were calculated.

The uptake and distribution of radioactive phosphorus within the tissues of *Ascaris lumbricoides* were examined by means of the autoradiograph technique. After incubation in media containing the radioactive phosphorus, the parasites were fixed in neutral 10% formol-saline and histological sections cut on a freezing microtome. The sections were then air-dried on microscope slides and autoradiographs prepared by placing the sections on fine grain X-ray film for 6–14 days according to the radioactive phosphorus content of the tissues. This procedure, selected because it would be least likely to disturb the distribution of radioactive phosphorus-containing compounds in the tissues, did not, of course, give satisfactory histological specimens, and when it was found that autoradiographs from frozen, air-dried sections were similar to those obtained by a rapid dehydration, paraffin-embedding process, the former method was discarded.

For the determination of total phosphorus (P_{total}) known volumes of perienteric fluid or known amounts of tissue were dried to constant weight at 104° C., ashed with sulphuric and nitric acids, and the phosphorus content estimated by the extraction method of Berenblum & Chain (1938).

PROCEDURE AND RESULTS

The uptake of radioactive phosphorus by Nippostrongylus muris

Infected rats of about 120 g. weight, which had been starved overnight, were lightly anaesthetized with ether and dosed with 0·5 ml. of a solution of disodium hydrogen phosphate containing approximately 2 microcuries of P^{32}. The solution was delivered directly into the oesophagus by means of a blunt, round-ended hypodermic needle. At intervals, rats were killed with chloroform; the worms were collected, washed repeatedly with saline, counted, and samples prepared for the estimation of radioactivity. As a rule, about 30 min. were occupied in washing the parasites from one rat. In each group of experiments the same period was spent washing each lot of parasites. The parasites were usually distributed in the intestine over the region 6–28 cm. from the pylorus. Accordingly, the samples of gut mucosa for P^{32} determination were taken from the intermediate points, 11 and 23 cm. from the pylorus. The samples were washed repeatedly with saline, excess fluid removed with filter-paper and the wet weight taken. The material was then ashed and samples were prepared for counting.

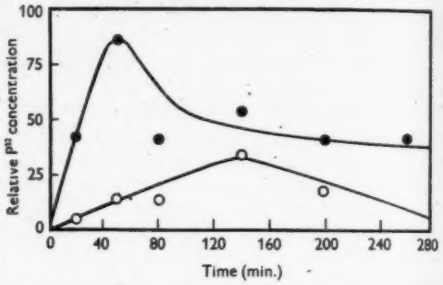

Fig. 1. Showing the relative amounts of radioactive phosphorus found in rat gut tissues (black dots) and *Nippostrongylus muris* (circles) at several periods after dosing infected rats *per os* with disodium hydrogen phosphate containing P^{32}.

The results obtained from one group of infected rats are shown in Fig. 1. The relative amounts of radioactivity found in host gut mucosa and *N. muris*, estimated on a wet weight basis, for periods up to 280 min. after dosing are shown. Results obtained with other groups of rats varied somewhat. Thus the largest amounts of P^{32} in the gut mucosa were found 45 and 50 min. after dosing, and maximum values for worms were recorded at 90 and 120 min. in different experiments. In two experiments the relative amounts of P^{32} in the host and parasites showed a relatively greater uptake than is illustrated.

The uptake of P^{32} by rat gut mucosa and *N. muris* after intramuscular injection of infected host animals, shown in Fig. 2, was determined as described for the previous experiment. Each injection contained approximately 3 microcuries of P^{32} given as disodium hydrogen phosphate in saline adjusted to pH 7·2. Results, similar to those shown in Fig. 2 for the P^{32} uptake by *N. muris*, were obtained in other experiments, though on one occasion a peak in the radioactivity of the gut mucosa was found 120 min. after injection.

It is clear from Figs. 1 and 2 that *N. muris* can take up inorganic phosphate rapidly from the host tissues.

The rate of uptake of P^{32} by host gut tissue shown in Figs. 1 and 2 is similar to that found by Cohn & Greenberg (1938).

The uptake of radioactive phosphorus by Ascaridia galli

Chickens of the same age, weighing about 350 g., which had been infected with *A. galli*, were starved overnight and then dosed with P^{32} directly into the lower oesophagus by means of a stiff catheter. The radioactive phosphorus was given as disodium hydrogen phosphate containing about 4 microcuries of P^{32} dissolved in 2 ml. of water for each bird. The chickens were killed at intervals after dosing, and the male and female parasites removed separately, washed repeatedly in saline, dried on filter-paper and weighed. Intestinal tissue samples, taken 30 and 45 cm. from the pylorus, were treated similarly. Samples for counting were then prepared as

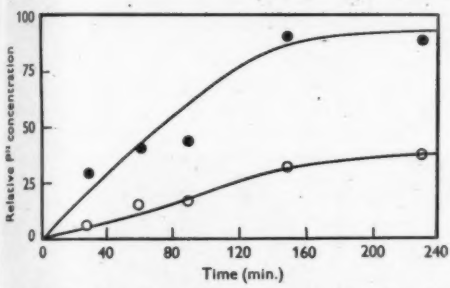

Fig. 2. Showing the relative amounts of radioactive phosphorus found in rat gut tissues (black dots) and *Nippostrongylus muris* (circles) at several periods after dosing infected rats intramuscularly with disodium hydrogen phosphate containing P^{32}.

described under 'Methods'. The uptake of P^{32} by female parasites and host gut, found in one experiment, is shown in Fig. 3. The radioactivity found in male parasites was similar to that shown for females, though the maximum uptake, which occurred 40 min. after dosing, was smaller.

The results obtained when this experiment was repeated were extremely variable. Peak values for P^{32} were obtained 20, 40 and 60 min. after dosing in different experiments. However, results were consistent in that a sharp maximum in radioactivity was obtained during the first hour. On some occasions male worms showed a higher radioactivity than females.

When 5 microcuries of P^{32} in saline were injected intravenously into infected birds, the radioactivity in the host gut, estimated as in the previous experiments, followed the course shown in Fig. 4. Radioactive phosphorus was never found in parasites from injected birds.

In contrast, then, to *Nippostrongylus muris*, *Ascaridia galli* did not appear to ingest host tissues. Inorganic phosphate, it would seem, was taken up at somewhat variable rates from fluids in the intestinal lumen and was rapidly lost by *A. galli*.

The uptake of radioactive phosphorus and its distribution in the tissues of Ascaris lumbricoides

Female *A. lumbricoides*, some of which had been ligatured at head and tail, were incubated at 37° C. in saline buffered at pH 6·8 and containing approximately 2·5 microcuries of P^{32}/10 ml. A slightly acid medium was selected as approaching the pH of pig small-intestine contents (Long & Fenger, 1917) from the region where *A. lumbricoides* is usually found.

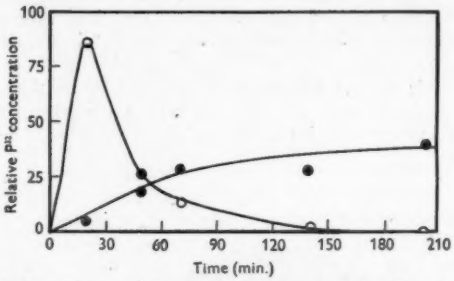

Fig. 3. Showing the relative amounts of radioactive phosphorus found in chicken gut tissues (black dots) and *Ascaridia galli* females (circles) at several periods after dosing infected chickens *per os* with disodium hydrogen phosphate containing P^{32}.

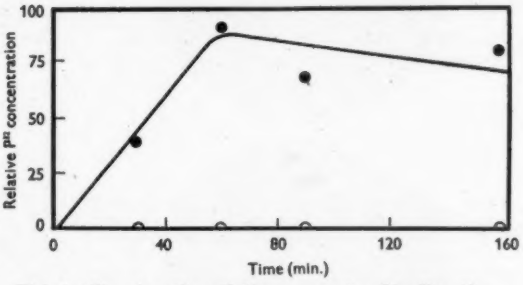

Fig. 4. Showing the relative amounts of radioactive phosphorus found in chicken gut tissues (black dots) and *Ascaridia galli* (circles) at several periods after dosing infected chickens intravenously with disodium hydrogen phosphate containing P^{32}.

Parasites were removed at intervals, washed quickly in warm saline, fixed, sectioned, and autoradiographs prepared (see 'Methods'). A number of autoradiographs, together with photomicrographs of corresponding histological sections stained with haematoxylin-eosin, are shown in Fig. 5.

After incubating for 1 hr. faint traces of radioactive phosphorus were found in the cuticle, in the tissues surrounding the lateral line organs, and in the tissues of the intestines of unligatured worms (see

Fig. 5*a*, *b*). In sections taken from anterior (Fig. 5*d*) and middle (Fig. 5*e*, *f*) regions of *A. lumbricoides*, the concentration of P^{32} in the intestinal tissues, relative to that in the other tissues, rose rapidly as the time of incubation increased. Faint autoradiographs were obtained from the female reproductive organs after long periods of incubation (8–20 hr.).

Autoradiographs obtained from sections prepared from ligatured parasites showed P^{32} in the cuticle and in the region of the lateral line. The radioactivity was not detected in other tissues (compare Fig. 5*c*, *d*).

From the results obtained it would appear that inorganic orthophosphate was absorbed almost exclusively from the alimentary tract of *A. lumbricoides* incubated *in vitro*. Although P^{32} entered the cuticle its passage thence to other tissues was not detected by the methods used in these experiments. The presence of P^{32} in the lateral line system, even after short periods of incubation, suggests that this organ may have functions other than excretory.

The P_{total} *of the tissues of* Ascaris lumbricoides

Table 1 shows the percentage dry matter and P_{total} found in four analyses. Figures for perienteric fluid refer to samples obtained from the pooled fluids of some five female worms. Other results were obtained from tissues of individual parasites. 'Ovary' refers to the fine convoluted tubules of the female organ. The muscle samples were contaminated with underlying hypodermis and also included parts of the lateral line and nervous systems. The bulk of these contaminants, however, was small compared to that of the muscle itself. 'Reproductive organ' refers to the whole female reproductive system, including the ovary, uterus and its contained eggs.

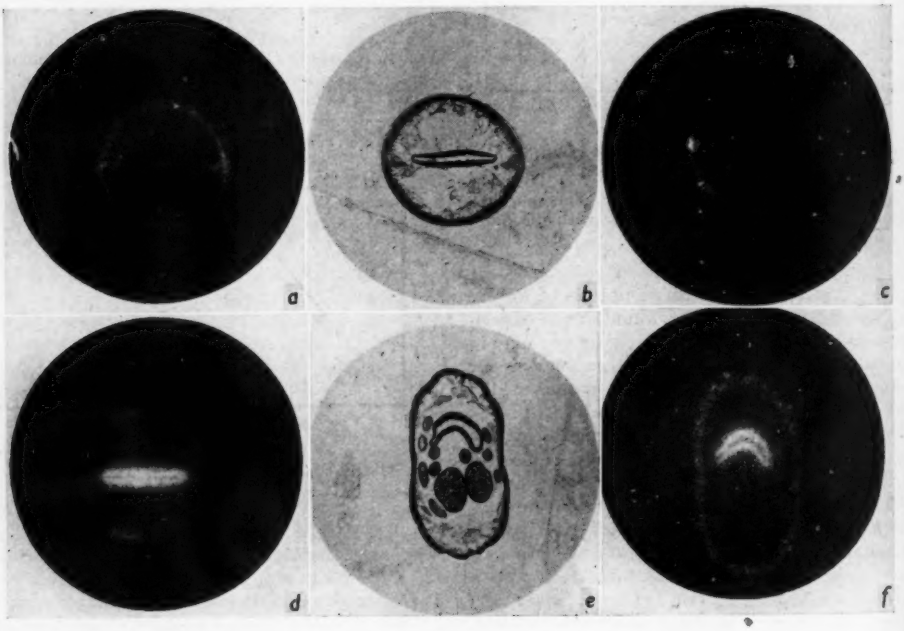

Fig. 5. (*a*) Autoradiograph of a section taken from the anterior end of an *A. lumbricoides* after incubating for 1 hr. in saline containing P^{32} as disodium hydrogen phosphate. (*b*) Photomicrograph of a histological section corresponding to (*a*), (*c*) and (*d*), and stained with haematoxylin-eosin. (*c*) Autoradiograph taken from a ligatured parasite after 2 hr. incubation. (*d*) Autoradiograph corresponding to (*c*) but taken from an unligatured worm. (*e*) Photomicrograph of a haematoxylin-eosin stained section corresponding to (*f*). (*f*) Autoradiograph from the middle region of unligatured *A. lumbricoides* incubated for 8 hr. All sections are enlarged × 5. For further explanation, see text.

Table 1. *The distribution of phosphorus in the tissues of* Ascaris lumbricoides

Tissue analysed	Percentage dry matter	mg. P/100 g. wet tissue
Muscle	19, 19, 20, 19	122, 122, 128, 109
Ovary	25, 24, 25, 23	187, 205, 193, 180
Reproductive organ	18, 19, 19, 18	194, 163, 149, 172
Alimentary tract	18, 18, 17, 19	213, 266, 246, 205
	g. dry matter/100 ml.	mg. P/100 ml.
Perienteric fluid	5·8, 6·0, 6·2, 6·1	51, 43, 47, 44

DISCUSSION

The distribution and amount of phosphorus in the tissues of *Ascaris lumbricoides*, as shown in Table 1, may not be that of the parasite in its natural environment. It has been found (Rogers, 1945) that the phosphorus content of the perienteric fluid changes as the worms age *in vitro*. Also, the total phosphorus of the intestine is dependent on the *in vitro* age of the worms and is influenced by conditions such as aerobiasis or anaerobiasis, under which the worms are kept. Nevertheless, the changes noted were small compared to the total amounts of phosphorus found in the present investigation, and it is probable that the results shown in Table 1 represent the phosphorus content of the parasites as they exist *in vivo*.

The phosphorus content of non-skeletal tissues of animals, particularly among invertebrates, varies from species to species, but in marine forms (see Vinogradov, 1944) it is generally higher than in *A. lumbricoides*. Marine vertebrates have a phosphorus content, nearer, though still somewhat higher, than that of the parasite, and terrestrial vertebrates, excluding their bony structures, usually contain much less. Thus, on a wet-weight basis, the human intestine (see Shohl, 1939) contains less than half, the uterus less than one-third, and blood serum about one-third of the amounts in the corresponding organs and perienteric fluid of *A. lumbricoides*.

If the relative activity of phosphorus metabolism of non-skeletal tissues is indicated by the total amounts of the element present it would appear that the ovary and intestine of *A. lumbricoides* are most important organs in this respect. Indeed, considering the marked synthetic activity and rapid phosphorus turnover taking place in the ovary, and the metabolism of the intestine being necessarily coupled with this, such a result might be expected. The muscles, on the other hand, are probably specialized more as storage than as locomotive organs, because radiographic studies with *A. lumbricoides* (human strain) have seldom revealed any movement within the host (Archer & Peterson, 1930), and though glycogen synthesis in the muscles is phosphorylative (Rogers, unpublished) the demand on phosphorus metabolism would not be as great as in the other organs.

The occurrence of comparatively large amounts of phosphorus in the 'cell-free' perienteric fluid is noteworthy and lends some support to Goldschmidt's (1906) contention that the so-called body cavity of *A. lumbricoides* is formed from the intracellular spaces of several large cells.

The results obtained in the present investigation suggest that inorganic orthophosphate enters *A. lumbricoides* entirely or predominantly via the alimentary tract. Though experimental conditions and the treatment of parasites during collection may have increased the permeability of the body wall it is unlikely that the permeability would have been decreased by such treatment. It should be noted that, in so far as phosphorylative activity was concerned, the muscular tissue taken from parasites which had been incubated for much longer periods than those used for these experiments was quite active and considerable phosphate transfer still took place.

The possible role of the lateral line system in the absorption of phosphate needs further examination.

The predominance of intestinal absorption for even such a simple molecule as orthophosphate in *A. lumbricoides* suggests that drug absorption by this route may be important in relation to anthelmintic efficiency.

If alimentary absorption is also important in *Ascaridia galli*, the observed differences in the rate of P^{32} uptake may have been due to varied feeding activities. The peak radioactivity values recorded during the hour following dosing *per os* would then be due to the uptake of host gut contents containing relatively large amounts of P^{32}, and the sudden fall (see Fig. 3) would indicate that a large proportion of phosphate passed directly through the parasite without absorption. This, together with the negative results obtained when P^{32} was injected intravenously into the host birds, suggests that *A. galli* feeds exclusively on gut contents, the phosphate passing rapidly along its alimentary canal with relatively poor absorption.

In contrast to *A. galli*, it would appear that *Nippostrongylus muris* fed actively on host tissue and showed a greater absorption of P^{32} (see Figs. 1 and 2). It is probable, therefore, that host gut

contents may be relatively unimportant in the diet of *N. muris* and if highly insoluble anthelmintics are taken up by parasites *per os* when they feed on the gut contents of treated host animals it would suggest that the feeding habits of *N. muris* may have some relation to its resistance to drug action (Rogers, 1944).

SUMMARY AND CONCLUSIONS

1. Compared with the mammalian intestine, uterus and blood serum, the corresponding organs and perienteric fluid of *Ascaris lumbricoides* were found to have a high phosphorus content. The muscle of the parasite, however, contained little more than half the phosphorus found in the mammalian muscle.

2. *A. lumbricoides* took up inorganic orthophosphate largely or exclusively through the alimentary tract. Small amounts were taken up by the cuticle and the lateral line system, but did not appear to pass on to other tissues.

3. *Nippostrongylus muris* in the intestine of the rat took up radioactive phosphorus which had been injected into the host intramuscularly. When the host was dosed *per os* the radioactivity appeared in the parasites at a lower concentration than in the host's small intestine.

4. *Ascaridia galli* failed to show any radioactivity when host birds were injected intravenously with inorganic orthophosphate containing P^{32}. A sudden rise and fall in the radioactivity of the parasites were found when host birds were dosed *per os*.

5. It is suggested that nematode parasites have a high demand for phosphate which they take up largely *per os*. *Ascaridia galli* feeds rapidly, but with poor phosphate absorption, on host gut contents. *Nippostrongylus muris* feeds on host tissues. It is suggested that the different feeding habits of *N. muris* and *Ascaridia galli* may have some relation to the efficacy of anthelmintics against these parasites.

The authors are indebted to Dr M. Kamen and Dr E. Reinhard of Washington University, St Louis, for a gift of radioactive phosphorus. Thanks are also due to Mr E. Parrish and Mr L. Turk of the McMaster Laboratory for their capable assistance.

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STUDIES ON THE NEMATODES PARASITIC IN THE LUNGS OF FELIDAE IN PALESTINE

By Ch. B. Gerichter, M.Sc., Ph.D., *Government Central Laboratories, Jerusalem* *

(With 27 Figures in the Text)

Six species of metastrongyloid nematodes have been recorded from the respiratory organs of Felidae: (1) *Aelurostrongylus abstrusus* (Railliet, 1898) Cameron, 1927, from *Felis catus*; (2) *Bronchostrongylus subcrenatus* (Railliet & Henry, 1913) Cameron, 1931, from *Felis pardus* and *F. tigris*; (3) *Metathelazia californica* Skinker, 1931, from *Puma concolor hippolestes*; (4) *Metathelazia felis* (Vogel, 1928) Dougherty, 1943, from *Felis pardalis*; (5) *Metathelazia massino* (Davtian, 1933) Dougherty, 1943, from *Felis catus*; (6) *Troglostrongylus troglostrongylus* Vevers, 1923, from *Felis bengalensis*. In the course of investigations on Palestinian Felidae one of these species, *Aelurostrongylus abstrusus*, was found in addition to two new species, *Troglostrongylus brevior* n.sp., and *Anafilaroides rostratus* n.g., n.sp.

Aelurostrongylus abstrusus (Railliet, 1898) Cameron, 1927

(Figs. 1–10)

Mueller (1890) described this species, from the lungs of a cat, under the name of *Strongylus pusillus*. Railliet (1898) proposed a new name, *Strongylus abstrusus*, as *pusillus* proved to be preoccupied. Kamensky (1905) transferred it to his newly proposed genus *Protostrongylus*. Railliet & Henry (1907), ignoring the latter genus, transferred the species to the genus *Synthetocaulus*. Cameron (1927*a*) found it necessary to create a new genus, *Aelurostrongylus*, for it and this status has been accepted by modern helminthologists.

Autopsy of seventy-three stray cats caught in Jerusalem showed that nineteen of them (i.e. 26 %) were infected with *A. abstrusus*. Cameron (1928) states that the adults are localized in the blood vessels of the lungs. He used to collect the specimens by ligating the vessels and subsequently opening them into a container. Hobmaier & Hobmaier (1935) did not succeed, however, in finding the worms in the blood vessels, but always found them in the parenchyma. The present author made several attempts to follow the technique of Cameron, but always with negative results. In all cases the worms were embedded in the parenchyma of the lungs. The diseased foci, whitish and swollen, reach 1–2 cm. in diameter, and are conspicuous by their state of hepatization. In addition to adult worms the tissue of these foci contains eggs in all stages of development, as well as first stage larvae. The adult worms are fragile and are so intimately embedded in the tissue that they usually break during extraction. The author was, however, successful in removing several score of unbroken specimens.

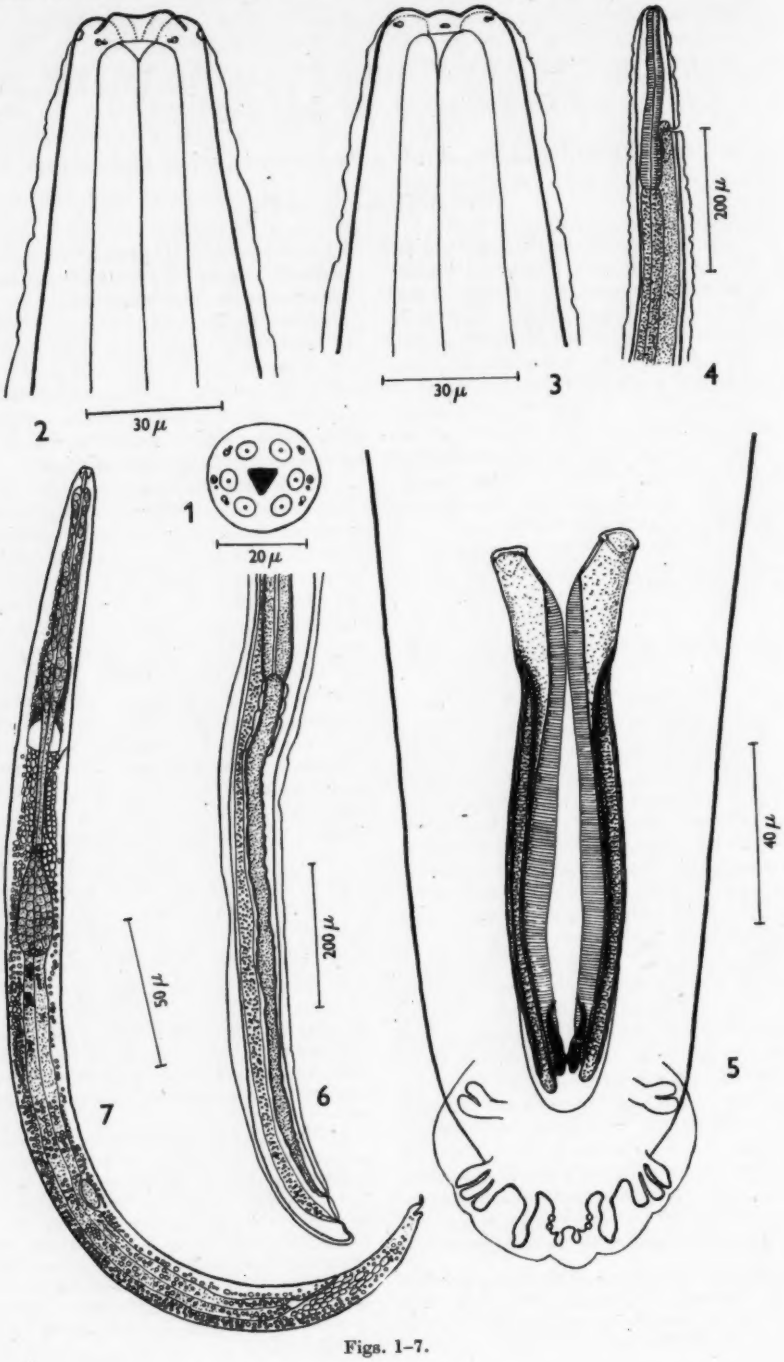
The worms are minute, thin, and enclosed in a teguminal sheath.† The oral opening is central in position and triangular in shape. There is no oral capsule. Around the oral opening (Fig. 1) there are six low perityls.‡ There is a minute papilla situated on the top of each perityl, and the six papillae constitute the internal circle of circumoral papillae. The external circle of papillae consists of four pairs of sublateral papillae and of two ventro-lateral papillae. All papillae are small, and in each of the sublateral pairs, the ventro-ventrals and the dorso-dorsals are smaller than the corresponding latero-dorsals and latero-ventrals. The ventro-laterals are so small that they may often be seen only with difficulty. Thus, there are a total of sixteen papillae in addition to two small amphids. The oesophagus is club-shaped. The excretory pore opens at the beginning of the last third of the oesophagus. The excretory gland is long.

Male. Length, 5·2–6·0 mm.; maximum width, 0·054–0·064 mm. Oesophagus, 0·24–0·27 mm. long

* This study was carried out in the Department of Parasitology, Hebrew University, Jerusalem. The author wishes to express his appreciation to Dr G. G. Witenberg for helpful advice and criticism.

† This term denotes a loose membranous sheath enveloping the whole body of the worm, firmly attached to it only around the oral, excretory and genital openings. The teguminal sheath occurs in all Metastrongyloidea, and, although not discussed by other authors, has been reproduced in accurately prepared drawings of several species.

‡ The author is indebted to Dr G. Witenberg for the suggestion of this term. It denotes immobile lip-like projections surrounding the oral aperture of nematodes, which take no part in the closing or opening of the oral aperture or in the process of taking food, and may, indeed, be situated at some distance from the oral aperture. It would therefore be misleading to call such projections 'lips'. In some nematodes they are developed in addition to true lips. They are not homologues of *pseudolabia* or *myolabia*. They occur in many Metastrongyloidea.



Figs. 1–7.

and 0·029–0·035 mm. wide. The dorsal ray (Fig. 5) is three times as wide as the other rays and it consists of a stout stem provided on its distal extremity with two finger-like appendages, in addition to two pairs of small sessile papillae situated at varying sites along the rim of the stem. It may be noted that in the drawing of Mueller (1890) the dorsal ray is depicted in the form of two separate branches forked at their ends, and in the drawings of Cameron (1927*a*) the digitiform appendages and papillae are not reproduced. The externo-dorsal rays are a little longer than other rays. All three lateral rays are separate and of equal length. The ventral rays are fused in their proximal halves. Spicules are equal in shape and size, and are 0·13–0·22 mm. long. They are cylindroid in their three distal fourths and widened at the proximal extremity, their inner surface is transversely striated, and their distal ends are joined by a common transparent membrane. The gubernaculum consists of two parallel, equal elements 0·018 mm. long, joined near their distal ends.

Female. Length, 10·0–10·4 mm.; maximum width, 0·08 mm. Oesophagus, 0·3 mm. long; maximum width, 0·032–0·040 mm. The terminal portions of the two uteri (Fig. 6) run parallel before joining the vagina. The latter is straight and is 0·78–0·80 mm. long. At the point of junction with the uteri there is a weak sphincter. There is no sphincter at the vulva. The distance from vulva to anus is 0·043–0·051 mm., the distance from the anus to the tip of the tail is 0·027–0·029 mm. Eggs measure 81–99 × 72–91 μ.

Several helminthologists have investigated the life history of *A. abstrusus*, and the most important observations have been made by Cameron on the one hand, and by Hobmaier & Hobmaier on the other. These authors came, however, to different conclusions. Cameron asserts that the mouse is the intermediate host, while the Hobmaiers found that land snails play this role, and that the mouse, as well as many other small animals, both warm and cold blooded, may serve as auxiliary intermediate hosts. The experiments and observations of the present author tend to support the Hobmaiers' view.

The first-stage larva (Fig. 7) is 360–390 μ long and 18–19 μ wide at the widest part of the body, near its middle. The narrow anterior extremity bears at least four small papillae and two amphids on its blunt tip. The oral opening leads into a short and narrow vestibule, and then into the rhabditoid oesophagus. The procorpus is comparatively wide, the metacorpus distinct, the isthmus narrow and the bulbus oval. The total length of the oesophagus is equal to almost half of the body length. The excretory opening is situated just behind the metacorpus, and a short, narrow excretory duct leads to it. The genital primordium is situated at the middle of the gut. The tail is 40 μ long, its tip is bent in the form of an S, and a short appendage is split off on its dorsal side. Except for slight individual variations the shape of the tip of the tail is constant.

Under experimental conditions separated larvae survive in tap water for as long as 7 weeks, although their activity slowly diminishes during this period. The following molluscs have been experimentally infected with the larvae of *A. abstrusus*: *Chondrula septemdentata*, *Helicella barbesiana*, *H. vestalis joppensis*, *Helix cavata*, *Levantina cesareana*, *L. hierosolyma*, *Monacha syriaca*, *Retinella nitellina*, *Theba pisana*, *Agriolimax* sp. and *Limax flavus*. Species of the genera *Helicella*, *Monacha* and *Agriolimax* proved appropriate intermediate hosts, while other species, though permitting complete development of the larvae, required longer periods.

The development of the larva to the second stage requires 11 days under optimum conditions. Larvae of this stage (Fig. 8) are 450–480 μ long and 31–34 μ wide. The anterior extremity is rounded and the oral capsule is very shallow (5 μ). The oesophagus is filled with large nuclei, the metacorpus is inconspicuous and the junction between the oesophagus and the gut divides the body in the proportions of 1 : 1½. The gut is wide and its large cells are filled with refractile granules. The tail is conoid and 30–33 μ long. The second stage lasts for 4 days and is followed by a 1 to 2-day lethargus. The third-stage larva (Fig. 9) is 460–510 μ long, and its maximum width is 26–28 μ. The oral opening leads into the oral capsule which is elongated in axial section. The relative size and the shape of the oesophagus are similar to those of the preceding stage, and it contains only a few granules. The gut also has lost most of its granules so characteristic of the preceding stage. The tail is 30 μ long, and ends in a small, globular appendage. The third stage lasts for 2 days, and the infective stage emerges without lethargus on the 18th day, under optimum conditions. The

Legends to Figs. 1–7.

Figs. 1–10. *Aelurostrongylus abstrusus*

Fig. 1. Circumoral structure, frontal view.
Fig. 2. Anterior extremity, ventral view, high magnification.
Fig. 3. Anterior extremity, lateral view, high magnification.
Fig. 4. Anterior extremity, lateral view, low magnification.
Fig. 5. Male bursa, ventral view.
Fig. 6. Posterior extremity of female, lateral view.
Fig. 7. First-stage larva.

infective larva (Fig. 10) is 520–550 μ long, and is encased in two sheaths, from which it escapes easily after a slight compression of the snail's tissue containing it, and shows great activity. In its internal structure it resembles the third stage, but is slimmer, and the posterior part of its oesophagus contains tightly packed granules.

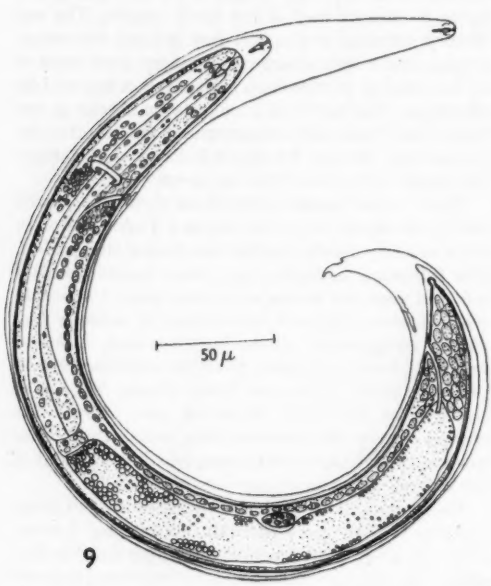


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Fig. 8. Second-stage larva.

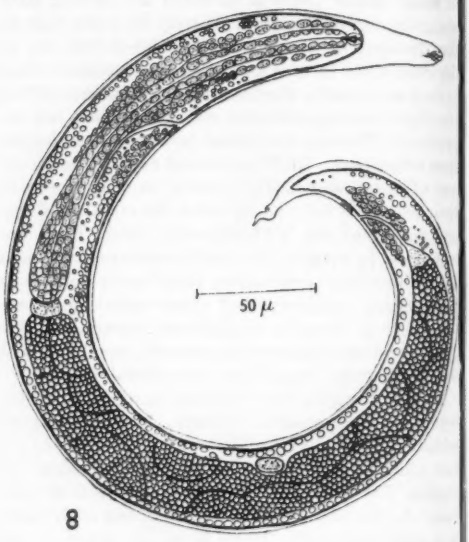

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Fig. 9. Third-stage larva.

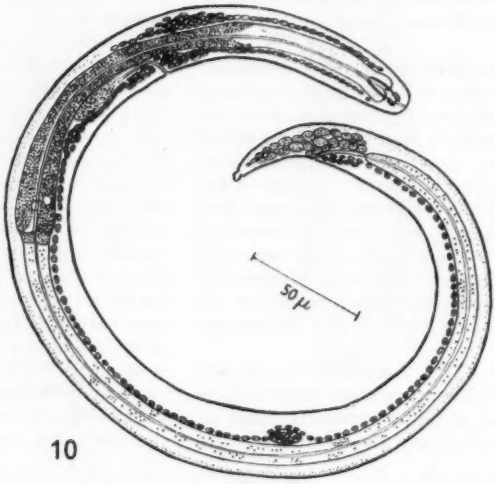

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Fig. 10. Infective larva.

The experiments were carried out at temperatures varying between 4° and 30° C. The optimum temperature for the development of the larvae in the mollusc is about 30° C. At lower temperatures development proceeds more slowly, and at temperatures below 8° C. it stops altogether, even in the best suited intermediate host. Infected molluscs were maintained at temperatures between 4° and 8° C., and no development took place for a period of 4 months, though the larvae retained the normal activity peculiar to the first stage. At this time part of them were transferred to 30° C., whereupon development was at once initiated and infective

larvae were found after 18–19 days. The remaining molluscs were left in the refrigerator as controls, and underwent no development during the three following months of the experiment.

Two suckling kittens were fed specimens of *Theba pisana* and *Levantina hierosolyma* containing infective larvae of *Aelurostrongylus abstrusus* and their faeces were subsequently examined every day. First-stage larvae appeared 39 days later. Seven weeks after the start of the experiment the lungs of the kittens were examined, and a heavy infection was found. It is noteworthy that no such heavy infection was observed in any of the nineteen cats found naturally infected. The lungs were dotted with numerous hepatized noduli 1 cm. and more in diameter, containing adult worms, eggs in all stages of embryonization, and first-stage larvae. Thirty-two adult specimens, all embedded in the parenchyma, were removed from the lungs of both cats.

Hobmaier & Hobmaier (1935), in their description of the development of the larva of *A. abstrusus* in molluscs, noted that the duration of the first stage is 10 days and that of the second stage 5 weeks or more. In the experiments of the present author, conducted at optimum temperature, the first stage lasted 11 days and the second 5 days. In this, as in related species, the first stage of larval development was always the longest. This was also true at lower temperatures.

The taxonomic position of the genus *Aelurostrongylus* has been a subject of controversy. In 1927 Cameron, who created this genus, placed it in the family Protostrongylidae. Skrjabin and Yershov (1933) regarded it as belonging to the subfamily Synthetocaulinae (synonym of Protostrongylinae), while Böhm & Gebauer (1934) ascribed it to the Metastrongylinae. Dougherty (1943, 1946) proposed to transfer it to the subfamily Filaroidinae, together with some other genera, all parasites of carnivores and possessing a well-developed male bursa, in spite of the fact that the more typical genera of Filaroidinae are characterized by the absence of a male bursa. In doing so Dougherty breaks with the orthodox view according to which the presence or absence of the bursa is of the highest taxonomic importance and according to which it has been customary to classify the genera on the basis of a gradual reduction of the bursa. Dougherty bases his opinion on the following arguments: the bursa of the second species of the genus *Aelurostrongylus*, *A. falciformis*, exhibits 'reduction of the bursa approaching that of *Filaroides*', and species of both genera occur in carnivores and use molluscs as intermediate hosts. The first argument is at variance with the existing description and drawing of *Aelurostrongylus falciformis* (Wetzel, 1938, p. 534, fig. 3*A*), which shows the bursa well developed and similar to that in *A. abstrusus*. Hence, the only morphologic ground is excluded. As to the other argument, it is irrelevant, for both carnivores and molluscs harbour species belonging to different subfamilies of Metastrongyloidea. In view of these facts it seems justified to leave the genus *Aelurostrongylus* in the subfamily Protostrongylinae.

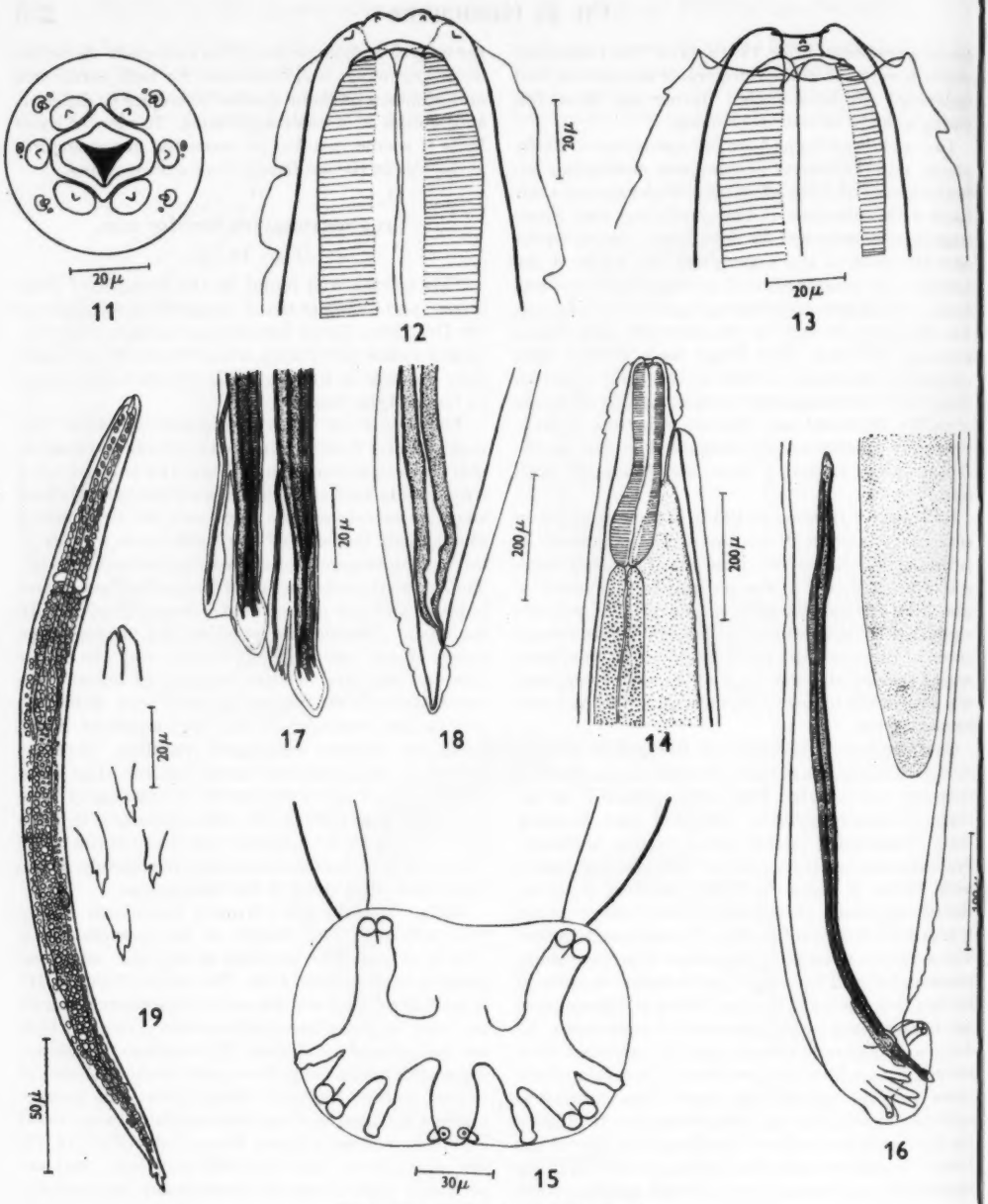
Troglostrongylus brevior n.sp.

(Figs. 11–22)

This species was found in the bronchi of *Felis ocreata* and *Catolynx chaus*, caught in the vicinity of the Dead Sea. In the former nine males and twenty-eight females were found, while the latter harboured only one pair of worms. *Felis ocreata* is considered to be the type host.

The body of the worm is stout and cylindroid. The oral opening is hexagonal with lateral short sides, and when seen from above (Fig. 11) it is surrounded by a double contour. There are six perityls of which the four sublaterals are large and the two laterals almost half the size of the sublaterals. There is a conspicuous papilla on each perityl (internal circle). The external circle consists of four pairs of sublateral papillae and one pair of ventro-lateral papillae. In each pair of sublateral papillae one is very large (latero-dorsal and latero-ventral) and the other (dorso-dorsal and ventro-ventral) is small. The ventro-laterals are discernible only with difficulty, and lie just ventrally to the large amphids. Thus, there are sixteen circumoral papillae. There is a shallow asymmetrical buccal capsule. The oesophagus (Fig. 14) is club-shaped. The unusually large excretory gland (Figs. 14, 16), extending in both sexes almost to the posterior extremity of the body, opens through a short tubule approximately at the level of the first third of the oesophagus.

Male. Length, 6·6–7·2 mm.; maximum width, 0·20–0·23 mm. The length of the oesophagus is 0·24–0·27 mm. The intestine is straight, and runs parallel to the genital tube. The bursa (Figs. 15, 16) is well developed and has an elongated-oval dorsal ray, with four papillae at its extremity, two of which are ventral and two lateral. The externo-dorsals are separated, while the postero- and medio-laterals are united, except for their distal parts. The antero-laterals are separated and the ventral rays are fused except for the distal third. The spicules (Figs. 16, 17) are equal, thin, and 0·51–0·60 mm. long, and are provided with a narrow transversely striated rim along almost their entire length. At the distal extremity each spicule gives off a long thin appendage, and its end bears four small digitiform appendages, all these structures being united by a thin membrane. The gubernaculum (Fig. 16) consists of an elongated stem, 0·078–0·081 mm. long, and two small ventrally bent side lobes between which the spicules slide.



Figs. 11–19. *Troglostrongylus brevior*

Fig. 11. Circumoral structure, frontal view.
Fig. 12. Anterior extremity, ventral view, high magnification.
Fig. 13. Anterior extremity, lateral view, high magnification.
Fig. 14. Anterior extremity, lateral view, low magnification.
Fig. 15. Male bursa, ventral view.
Fig. 16. Male bursa, lateral view.
Fig. 17. Tips of the spicules.
Fig. 18. Posterior extremity of female, lateral view.
Fig. 19. First-stage larva.

Female. Length, 9·6–16·8 mm.; maximum width, 0·26–0·40 mm. The intestine and genital organs are twisted. The vulva is slightly post-equatorial. The tail is conoid, 0·09–0·10 mm. long. Eggs measure 81–90 × 50–62 μ and contain a developed larva.

The generic position of this species was determined on the basis of its similarity to the type* species of the genus, *Troglostrongylus troglostrongylus* Vevers, 1923. These species differ in the size of the worms, the length of the spicules and gubernaculum and in the pattern of the lateral bursal rays. The size of *T. brevior* is about half that of the type species. Its spicules and gubernaculum are about a quarter of the length (0·51–0·60 and 0·078–0·081 compared with 2·25 and 0·27 respectively). In *T. brevior* the medio-lateral bursal rays are partly fused with the postero-lateral rays, while in *T. troglostrongylus* they are fused with the antero-lateral rays.

The first-stage larvae (Fig. 19) occur in the faeces of infected animals. They are 300–310 μ long and 17 μ wide. The oral opening is slightly dorsal to the anterior tip of the body. There is a small oval oral cavity. The rhabditoid oesophagus occupies a little less than half of the body length. Its walls contain numerous large nuclei. The thin excretory duct opens behind the level of the nerve ring, i.e. behind the middle of the oesophagus. The intestinal cells are filled with food granules. The genital primordium is situated at the middle of the intestine. The tail is 33–42 μ long, and has a deeper dorsal incision and a shallower ventral one near its tip. Slight individual variations are observed in the shape of the caudal end.

In order to isolate first-stage larvae uteri containing ripe eggs were placed in tap water. On the following day the larvae hatched and were collected. Such larvae are very active, but their vitality diminishes during the following days and some of them die by the end of a week. On the 20th day (at 16° to 20° C.) most of the larvae are dead, and after 4 weeks no living larvae remain. During all this time the larvae undergo no change and this suggests that an intermediate host is necessary for further development.

The following molluscs have been artificially infected at temperatures varying from 4° to 27° C.: *Chondrula septemdentata, Helicella barbesiana, H. vestalis joppensis, Monacha syriaca, Retinella nitellina, Theba pisana, Limax flavus.*

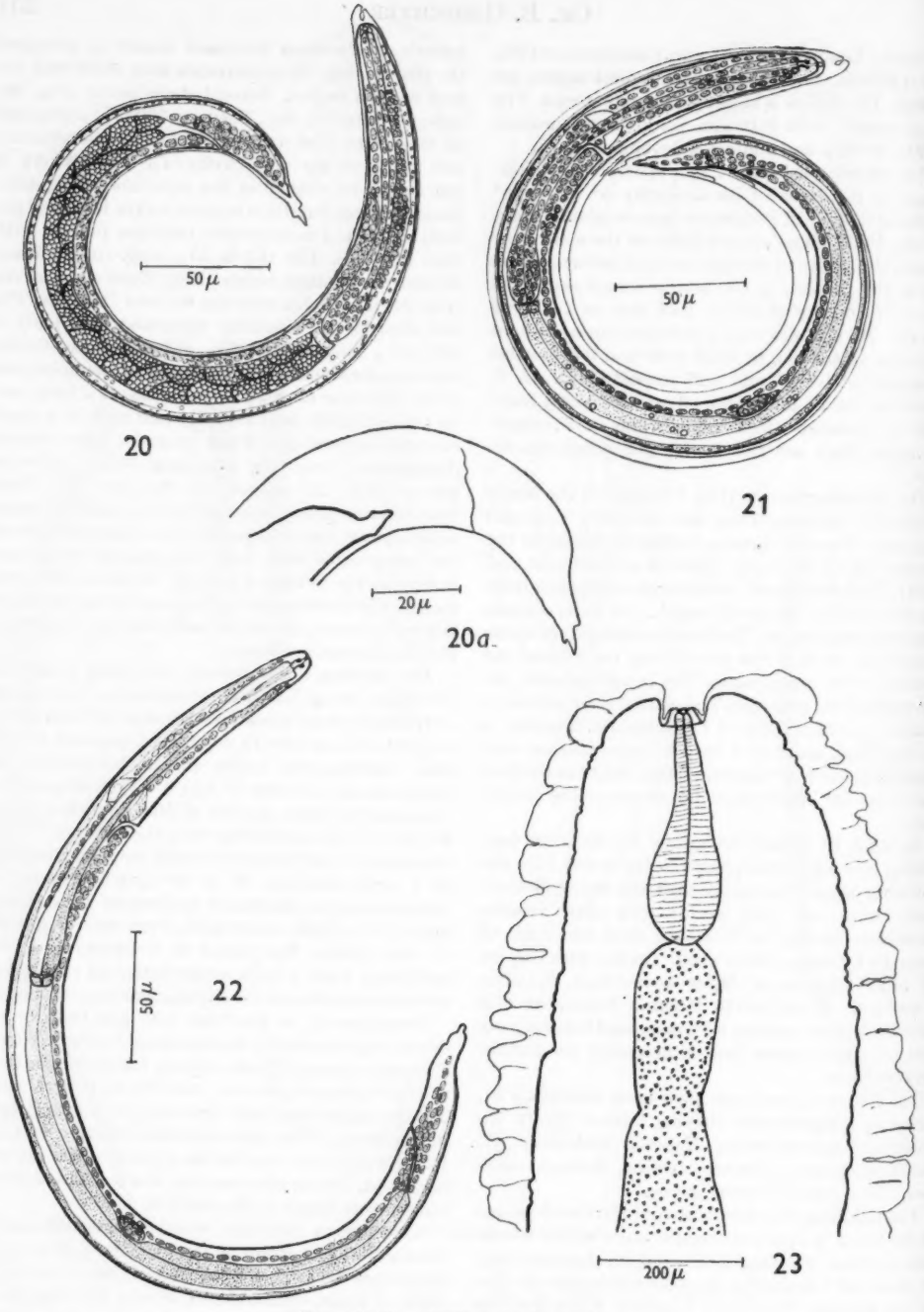
The following is a description of the development of the larva at optimum temperature and in a suitable mollusc. First signs of larval development may be observed 2 days after its penetration into the foot of the mollusc. The larva becomes wider and the intestinal cells show increased masses of granules. On the 4th day the movements slow down and the first ecdysis begins. Second-stage larvae (Fig. 20) appear on the 5th day. They are 330–370 μ long and 22–24 μ wide. The oral opening is situated centrally and leads to an oral cavity 4 μ long. There is practically no change in the structure of the oesophagus, whose length in relation to the length of the body is 1 : 3. The intestinal cells are packed with food granules. The tail is 35 μ long, its tip being delimited by a slight constriction. Third-stage larvae (Fig. 21) appear between the 6th and 7th days. The two sheaths are distinctly separated. The body is 420–450 μ long and 19–20 μ wide. The oral cavity has sclerotized walls. The oesophagus resembles that of the previous stage. The tail is 38–42 μ long, and its tip is slightly bent dorsally and ends in a small rounded appendage. Food granules have almost disappeared from the intestinal cells. Infective larvae (Fig. 22) appear on the 8th day. They resemble the previous stage but are slightly longer and slimmer, the large nuclei have disappeared from the oesophagus wall, and fine granulation is seen chiefly in the posterior part of the subventral segments. The sheaths are brittle, and the larva, which is very active at this stage, easily escapes from them at the slightest pressure.

The quickest development, occupying 8 days, as described above, has been observed in both species of *Helicella* at temperatures between 22° and 27° C. In *Monacha syriaca* 15 days were required for the same development under similar conditions. At temperatures between 4° and 8° C. development is completed in both species of *Helicella* after about 40 days. It is noteworthy that the larva of *Troglostrongylus brevior* is able to reach the infective stage at a temperature of 4° to 8° C., while larvae of *Aelurostrongylus abstrusus* underwent no development under these conditions, even after 7 months of observation. The period of development in the molluscan host, 8 days under optimum conditions, is the shortest known for any metastrongylid species.

Development in the final host has been reproduced experimentally in a suckling domestic kitten. Two specimens of *Theba pisana*, harbouring a total of seven infective larvae, were fed to it with milk. Twenty-eight days later first-stage larvae appeared in the faeces. Fifty days after the infective feed, i.e. 22 days after the first larvae appeared, the cat was sacrificed. Seven adult worms, five females and two males, were found in the small bronchi.

In order to establish whether *Troglostrongylus brevior* is able to infect an auxiliary host, four white mice were fed four or five infective larvae each. In three of them, examined 10, 30 and 120 days later, respectively, no larvae were found in the internal organs or in the musculature. In the fourth mouse, examined 120 days after the day of infection, an

* There is another species, *T. delicatus* Travassos, 1946, *Rev. Brasil. Biol.* 6, p. 499, from *Didelphis.*



Figs. 20–22. *Troglostrongylus brevior*

Fig. 20. Second-stage larva. Fig. 21. Third-stage larva. Fig. 22. Infective larva.

Fig. 23. *Anafilaroides rostratus*. Anterior extremity.

infective larva was found encysted on the surface of the lung. It should be pointed out that owing to the small number of larvae used in this experiment they may easily have escaped observation, and it may be presumed that other mice harboured them as well. Thus it may be accepted that *T. brevior* may infect an auxiliary host as does *Aelurostrongylus abstrusus*, as demonstrated by Hobmaier (1937).

TAXONOMIC POSITION OF THE GENUS *TROGLOSTRONGYLUS*

The genus *Troglostrongylus* belongs to a group of seven metastrongylid genera (*Bronchostrongylus*, *Crenosoma*, *Dictyocaulus*, *Heterostrongylus*, *Otostrongylus*, *Skrjabingylus* and *Troglostrongylus*) in which the vulva is situated near the middle of the body, in contrast to other genera in which the vulva is situated near the posterior end. This peculiarity was the cause of a controversy relating to the taxonomic position of the seven above-mentioned genera which was introduced mainly by Dougherty (1945). In his radical criticism of the classification of the seven genera listed above, Dougherty proposed to transfer all of them from the Metastrongylidae to Trichostrongylidae solely on the morphological basis of the structure of the vagina, in which this author sees homologies with the vagina of Trichostrongylidae, and on the basis of the equatorial position of the vulva. One of these genera, *Dictyocaulus*, was separated from the remaining Metastrongyloidea as early as 1933 by Skrjabin & Yershov, who placed it in a distinct subfamily, Dictyocaulinae, because of its developed double dorsal rays. In 1941 Skrjabin proposed to transfer this subfamily to the Trichostrongyloidea because of its direct mode of development. The biological and morphological characters of the genus *Dictyocaulus* make its position peculiar among Metastrongyloidea, but this question will be discussed in another paper. The position of the remaining six genera have to be discussed here. The author is of the opinion that Dougherty's arguments are insufficient to justify the transfer of these genera to the Trichostrongylidae, especially in the face of other arguments which emphasize their relationship to Protostrongylinae (Metastrongylidae), such as (1) similarity of the morphology of the larvae, (2) similarity of the life histories, (3) pattern of the circumoral papillae, and (4) reduction of the dorsal ray.

Troglostrongylus brevior and *Aelurostrongylus abstrusus* may serve as an example of the similarity between the larvae of all stages. In spite of the varying positions of the vulva in these species, the remarkable similarity of their larvae doubtless indicates their close phylogenetic relationship. In three of the mentioned genera, whose life histories are known, *Crenosoma*, *Skrjabingylus* and *Troglostrongylus*, molluscs serve as intermediate hosts, as they do in other Protostrongylinae whose life histories have been investigated. As for the pattern of the circumoral papillae, known only in the latter three genera, sixteen in number, it is identical with those in other protostrongyline genera, in which this pattern is known. The tendency towards reduction of the dorsal ray, which is represented by one stem only in all six genera, and in *Skrjabingylus* occurs in the form of a rudiment, is common to all Protostrongylinae and has no counterpart in the Trichostrongyloidea.

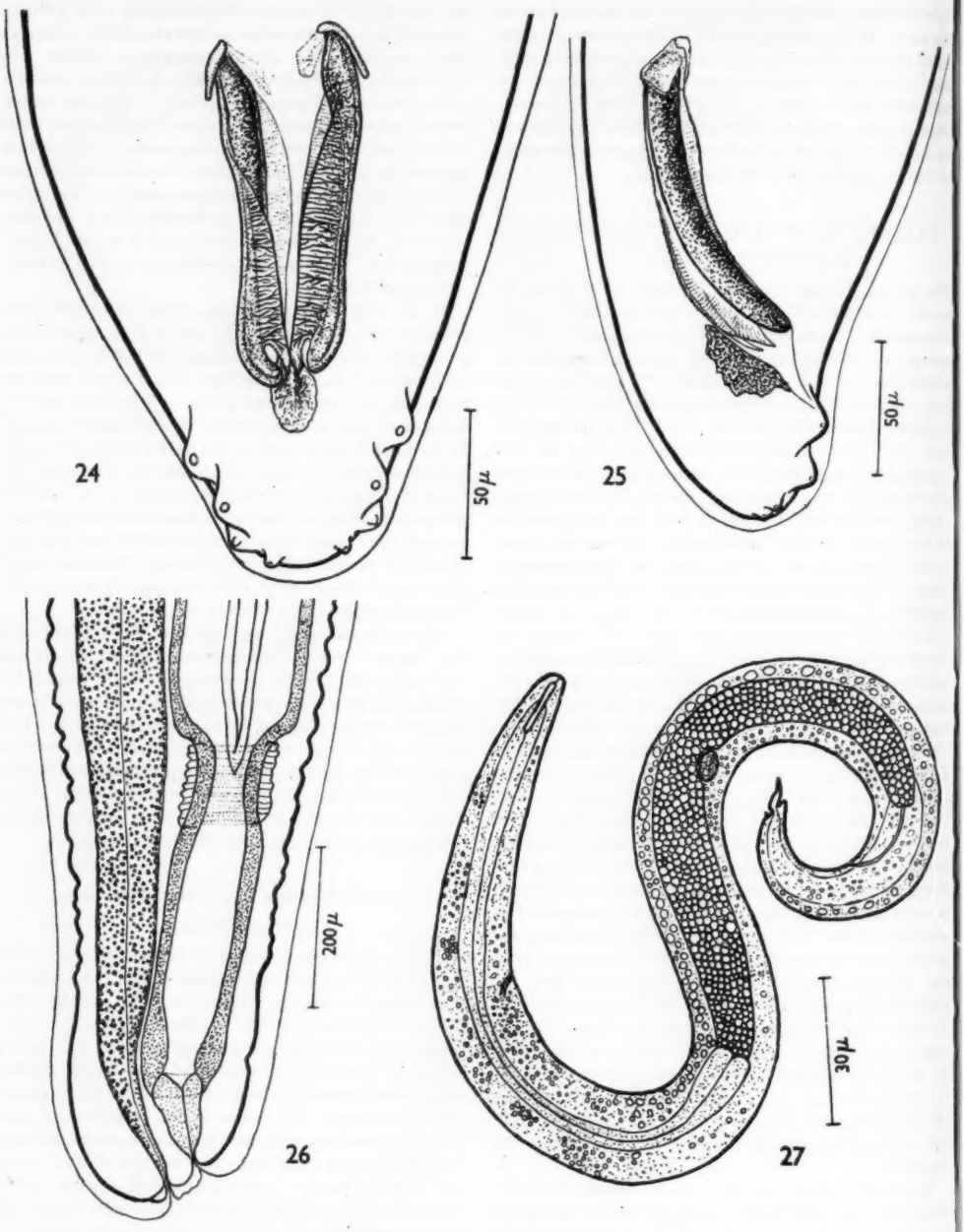
It is thus seen that the single morphological character emphasized by Dougherty is opposed by a complex of characters closely correlated with one another. The shifted position of the vulva may be regarded not as an expression of phylogenetic relationship but as a secondary evolutionary feature. Whereas it is true that its occurrence in the genera in question is conspicuous, and may serve as a convenient character for uniting them in a distinct group within the subfamily, it can never be sufficient ground for transferring them to a different family. It may be recalled that in the family Trichostrongylidae, *sensu* Dougherty, there are genera with vulva in both terminal and equatorial positions.

As for the alleged homologies in the structure of the vagina between the genera in question on the one hand, and the Trichostrongylidae on the other, the author prefers to reserve judgement until more detailed embryological studies are available. For the present, the somewhat peculiar structure of this organ in the genera with an equatorially situated vulva is regarded as a function of the position of the vulva, and as such it does not bear on the higher taxonomy of the genera in which it occurs.

Anafilaroides n.g., **rostratus** n.sp.

(Figs. 23–27)

This species was found only once in the course of the dissection of seventy-three cats in Jerusalem. The worms were embedded in the parenchyma of the lungs, and were visible under the pleura to the naked eye. The worms were so intimately associated with the tissue of the host that they broke during extraction, and only one male was secured uninjured. Only fragments of females could be collected, but this material was sufficient for the reconstruction of the anatomical structure. The worms of both sexes are covered with a wide teguminal sheath which forms numerous folds. The oral opening is triangular and is situated on the top of a rostrum-like structure which may be either extruded or withdrawn. There is no buccal cavity. The oral opening leads directly into the oesophagus (Fig. 23), which has the shape of a club, its posterior part being four times as wide



Figs. 24–27. *Anafilaroides rostratus*

Fig. 24. Posterior extremity of male, ventral view.
Fig. 25. Posterior extremity of male, lateral view.
Fig. 26. Posterior extremity of female, lateral view.
Fig. 27. First-stage larva.

as the anterior portion. Its internal structure is uniform. No deirids were found. The intestine is intertwined with the genital tubules in the female, and is straight in the male.

Male. Length, *c.* 30 mm.; maximum width, 0·24–0·29 mm. Its posterior extremity is straight. The length of the oesophagus is *c.* 0·27 mm. There are no bursa or rays. Four pairs of postanal papillae are situated laterally on the tail (Figs. 24, 25), the last pair being the smallest. The spicules are equal, stout, and slightly curved ventrally, are provided with three longitudinal alae and terminate in three small appendages. The length of the spicules is 0·11–0·12 mm. The gubernaculum is single, almost oval, of spongy consistency, and 0·04–0·05 mm. long.

Female. The length is presumably 50·0 mm.; maximum width to 0·69 mm. The oesophagus is 0·29 mm. long. At the junction of the uteri there is a weak sphincter. The vagina (Fig. 26), 0·58–0·65 mm. long, is provided with a sphincter-like structure at its distal end. The vulva is situated almost immediately in front of the anus on the ventral side of the rounded posterior extremity, and the tail is thus practically not discernible. The uteri contain eggs and free larvae. The ova in utero are 80–90 μ long.

First-stage larva (Fig. 27): length 300–320 μ; maximum width 17–18 μ. The oral opening is situated centrally and leads through a short passage into a cylindroid buccal cavity which is 10–12 μ long, its posterior half being inserted into the oesophagus. The latter is rhabditoid, and a little less than half the length of the body. The intestine is filled with food granules. The length of the tail is 31–35 μ. The tip of the tail has a fixed shape: it has a deeper notch on the ventral side and a shallower one on the dorsal side, so that it looks like a small undulating appendage. At the proximal edge of the dorsal notch there is a minute spine.

Owing to the limited material at our disposal only a few specimens of three species of snails were artificially infected: *Helicella vestalis joppensis*, *Monacha syriaca* and *Theba pisana*. The jars containing the infected snails were kept outdoors, where the temperature was 4·8°–12·5° C. (December). After 26 days one specimen of *Theba* was found to harbour larvae of *Anafilaroides*, which had slightly progressed in their development, being before the first ecdysis, 475 μ long and 30 μ wide. Ten days later a specimen of *Monacha* was found to contain larvae at the same stage of development. Though these experiments cannot be regarded as conclusive, the slight advance in the internal organization of the larvae proves that the latter had proceeded on their normal course of development. The slow rate of development may be explained by the low temperature at which the experiment was conducted.

The genus *Anafilaroides* closely resembles the genus *Filaroides*, the difference between them consisting mainly of: (1) the different shapes of the spicules, which in the new genus are straight instead of sickle-shaped; and (2) the relative position of vulva and anus, which in the species of *Filaroides* are situated on the ventral side of the body at some distance from each other and from the tip of the tail, while in the new genus they are situated close to each other almost at the rounded extremity of the body.

Diagnosis: Filaroidinae. Teguminal sheath wide. Oral opening triangular, situated at the tip of a small, extrusible, rostrum-like structure. Oesophagus club-shaped, of homogeneous structure. Deirids not evident. Sexual dimorphism conspicuous. Male: posterior extremity straight. No bursa or rays; postanal genital papillae present. Spicules stout and straight. Gubernaculum single, compact. Female: vagina comparatively short. There is a sphincter at the junction of the uteri and a sphincter-like structure at the vaginal opening. Vulva close to anus, both opening near the posterior extremity of the body. Viviparous. Development in molluscs.

Type species: *Anafilaroides rostratus* n.sp.

SUMMARY

Three species of lung nematodes have been found in Felidae in Palestine: *Aelurostrongylus abstrusus*, *Troglostrongylus brevior* n.sp. and *Anafilaroides rostratus* n.g., n.sp. The life histories of all these species have been studied. Their larvae develop in terrestrial molluscs. Some new observations on the factors influencing the development of the larvae of *Aelurostrongylus abstrusus* are described. The whole developmental cycle of *Troglostrongylus brevior* has been experimentally reproduced. Initial stages of the development of the larva of *Anafilaroides rostratus* in molluscs have been observed. The taxonomic position of the genera *Aelurostrongylus* and *Troglostrongylus* is discussed and their protostrongyline character is demonstrated.

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THE DIFFERENTIATION AND NOMENCLATURE OF *LEISHMANIA*

By R. KIRK, M.D., *Stack Memorial Research Laboratories, Khartoum, Anglo-Egyptian Sudan*

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I. INTRODUCTION

The genus *Leishmania* was created by Ross for the parasite of Indian kala azar which is now generally known as *Leishmania donovani* (Laveran & Mesnil, 1903). The characters of the genus were later defined by Wenyon (1913) in a scheme of classification of trypanosomes and allied flagellates. Wenyon separated flagellates of the genus *Leishmania* from those of the genus *Herpetomonas* by the absence of crithidia and trypanosome forms, and from those of the genus *Leptomonas* on the grounds that *Leishmania* have both a vertebrate and invertebrate host and 'in all probability will be found to have lost the encysted stage...by which transmission is effected in the true insect flagellates having no developmental stages in a vertebrate'.

The life history of *Leishmania* was then unknown. The possible role of sandflies in transmission had been suggested by Pressat (1905), the Sergents (1905) and Wenyon (1911 *a*, *b*), but various other suggestions had also been made. Over 30 years elapsed before Adler & Ber (1941) and Swaminath, Shortt & Anderson (1942) proved conclusively that *Phlebotomus papatasii* and *P. argentipes* can transmit, respectively, oriental sore and kala azar to man by the bite. During the intervening years a great deal of evidence accumulated to incriminate sandflies as vectors in different places, but in the absence of final proof many workers were unwilling to accept this conclusion, and there always remained a possibility that transmission in nature might ultimately be shown to occur in other ways. However, there can now be little doubt that the species of *Leishmania* of man and dogs are normally transmitted in nature by insects of the genus *Phlebotomus*. Also, a great deal of observation and experiment has now confirmed that there is no encysted stage in the life cycle of *Leishmania*.

While the characters of the genus have been established on a scientific basis there has been much argument about the separation of species of *Leishmania*. Here the criteria most commonly accepted are purely medical and are provided by the clinical features of the case from which the parasite was obtained. Not only are the so-called species indistinguishable morphologically, but attempts to differentiate them by the techniques of bacteriology have given conflicting results and there is no general agreement that they can be so differentiated. Systematists have long regarded this as unsatisfactory and have argued that all forms of human leishmaniasis are caused by infection with one and the same species of parasite. While there are many arguments in favour of this view, the purpose of this paper is to show that the different clinical types of human leishmaniasis are correlated with biological differences in the causal parasites.

II. EARLY HISTORY AND NOMENCLATURE

The first observer to report protozoal parasites in human leishmaniasis was Cunningham, who in 1885 described 'peculiar parasitic organisms' in sections of a Delhi boil fixed in alcohol and stained with gentian violet. Owing to the crude technique employed the leishmania showed no structure and Cunningham misinterpreted their nature, regarding the host macrophage as an amoeboid parasite and the leishmania within it as spores. His observations were confirmed by Firth (1891), who suggested the name '*sporozoa furunculosa*, to indicate the peculiar pathological influence of the parasite'.

In 1898 Borovsky published the result of his researches on 'Sart sore' and independently described, but did not name, the parasite now known as *Leishmania tropica*. Borovsky evidently knew nothing about the publications of Cunningham and

Firth, but recognized the parasites as protozoa and gave a more accurate account of them in relation to the elements of the tissues of the host than his predecessors, including a description of the nucleus and rod-shaped kinetoplast.

In 1900 Leishman observed the small oval bodies now known as *Leishmania* in the spleen of a soldier who had died of an obscure Indian disease called Dum-Dum fever. He did not immediately publish this observation, but later, while studying experimental trypanosomiasis in rats, noted the morphological similarity between these bodies and trypanosomes and surmised that they might be degenerative forms of a trypanosome. In 1903 he finally published his observations in a paper entitled 'On the possible occurrence of trypanosomiasis in India'. In the same year Donovan (1903) independently reported the finding of similar bodies in spleen punctures from cases of Dum-Dum fever in India. There was much discussion as to the nature of the Leishman-Donovan bodies, as they were called. Laveran regarded them as piroplasms and, with Mesnil, suggested the name *Piroplasma donovani*, while others regarded them as trypanosomes. Ross (1903) interpreted the findings in the same way as Cunningham had done with the parasite of oriental sore. He regarded the host cell as the parasite and the enclosed *Leishmania* as spores. He referred the parasite accordingly to the Sporozoa, emending Laveran and Mesnil's name to *Leishmania donovani*, in honour of the two workers who had independently discovered and described the parasite.

Meanwhile Wright (1903) in Boston observed peculiar bodies in a tropical ulcer occurring in a child who had come from Armenia, and proposed the name *Helcosoma tropicum*, suggesting that the parasite was a protozoon allied to the microsporidia. Marzinowsky & Bogrow (1904), working in Russia, found a similar organism in a case of oriental sore in a boy who had lived in Persia, and proposed the name *Ovoplasma orientale* for the parasite. In a review of Wright's paper Mesnil (1904*a*) stated that parasites identical with those described by Wright had already been seen by him in 1903 in preparations from a case of oriental sore received from Marzinowsky, and pointed out the resemblance between these parasites and *Piroplasma donovani* Laveran & Mesnil, the recently discovered parasite of Indian kala azar. The morphological similarity between the two parasites was confirmed by Leishman (1904*a*) and Christophers (1904), the former maintaining his belief in the flagellate nature of the organism, the latter regarding it as one of the microsporidia. The subject was reviewed by Blanchard (1904), who concluded that there was no difference between the parasites of oriental sore and those of kala azar. He accordingly referred the former to the same genus as the latter, under the name *Leishmania furunculosa* (Firth, 1891), admitting, however, that he agreed with Leishman's view that the parasites might be degenerative stages of a trypanosome.

The subject was clarified when Rogers (1904) succeeded in cultivating the parasite of Indian kala azar and demonstrating its flagellate stage, which he at first considered to be a trypanosome. At a meeting of the British Medical Association in London, Leishman (1904*b*) compared the flagellates to the insect parasite *Herpetomonas muscae-domesticae*. Mesnil (1904*b*), reviewing this discussion and the paper by Rogers (1904) on the cultural forms, pointed out that these were more like *Crithidia* or *Herpetomonas* than *Trypanosoma*. In a later paper Rogers (1906) concluded that the parasite of kala azar was a *Herpetomonas*, and proposed that it should be renamed accordingly, a suggestion which Mesnil (1906) formally endorsed in altering the name to *Herpetomonas donovani* (Laveran & Mesnil, 1903). A few years later Mesnil (1909) again changed the name of this parasite to *Leptomonas donovani* (L. & M.).

Meanwhile Patton (1909) independently concluded that the correct name of the parasite of kala azar was *Herpetomonas donovani*, and in conformity with this transferred the parasite of oriental sore to the same genus as *Herpetomonas tropica*. In a later paper Patton (1922) decided that Firth's nomenclature should be revived as valid, and proposed the name *Herpetomonas furunculosa* for the parasite of oriental sore. Other names suggested for this parasite include *Leishmania wrighti* (Nicolle, 1908), *Crithidium cunninghami* and *Leishmania cunninghami* (Carter, 1909) and *Plasmosoma jerichaense* (Huntemuller, 1914). As Hoare (1938) points out the first three of those names were introduced with utter disregard of prior claims, but Wenyon (1926), who examined Huntemuller's films, containing bodies which the latter saw in an oriental sore and named *Plasmosoma jerichaense*, concluded that these bodies were badly stained *Leishmania tropica*.

The first author to adopt the modern name *Leishmania tropica* (Wright, 1903) for the parasite of oriental sore was Lühe (1906), who rejected Firth's name *Sporozoa furunculosa* on account of its plural form. Lühe appears to have been better acquainted with the rules of nomenclature than with *Leishmania*, for he includes these parasites with the piroplasms, in spite of all the evidence which had accumulated in favour of their flagellate nature.

III. DIFFERENTIATION OF *LEISHMANIA*

Morphology and cultural characters

Some workers (Brumpt, 1936) have reported differences in size between *L. tropica* and *L. donovani*. Yakimoff (1915), studying *L. tropica* in Turkestan, believed he could differentiate two varieties,

L. tropica var. *major* and *L. tropica* var. *minor* on morphological grounds, var. *major* being larger than var. *minor* and having a different geographical distribution. Large forms of *L. tropica* have been noted in mice (Adler, 1929), and spermophils (Caminopetros, 1936). Hindle (1930) studied a strain of *L. donovani* from China in which the cultural forms were abnormally small. When isolated by Dr Young in Peking this strain was normal, but in the course of repeated subcultures the flagellates had become modified into small forms. Adler had found that this modified strain did not return to normal on any medium, and passage through *Phlebotomus papatasii* was also without effect. Hindle concluded that it was much less pathogenic to hamsters than the normal cultural forms of Chinese kala azar. Microscopically, however, he could find no difference between the leishmania forms in a hamster which became infected with this strain and those found in other hamsters inoculated with ordinary strains of Chinese kala azar. Developmental forms have occasionally been reported in the vertebrate host. Escomel (1913) saw forms with short flagella in cases of cutaneous infection in Peru, and named them *Leishmania americana* var. *flagellata*. The significance of these minor differences in size and shape, both in the vertebrate host and in culture, is at present uncertain, but in a recent paper Adler (1947) concludes that *L. donovani* can be distinguished from *L. tropica* by the relatively larger size of the leishmania forms of the latter in the tissues of inoculated hamsters.

Mayer & Malamos (1936) and others before them believed they could distinguish *L. tropica* from *L. donovani* by the type of growth on Nöller's medium, but found that a strain of *L. tropica* which had been repeatedly passaged in mice, causing visceral infection in these animals, developed the cultural characters of *L. donovani*. Other workers (Chodukin, Sofieff & Kevorkov, 1935) found that it was not possible to differentiate *Leishmania* by the appearance of cultures on Nöller's medium. Noguchi (1924), Kligler (1925), Cleveland & Collier (1930), and Chodukin *et al.* (1935) attempted to differentiate *Leishmania* by the use of culture media containing various carbohydrates, but found this impossible. More recently, Senekjie & Zebouni (1941) found fermentation tests of no use in the differentiation of *Leishmania*, but believed they could distinguish *L. brasiliensis* from *L. infantum* (*donovani*) and *L. tropica* by its resistance to bile, which causes rapid lysis of *L. tropica* and *L. infantum*. Adler (1947) believes that *L. donovani* and *L. tropica* can be distinguished by the character of the culture in Locke-serum-agar when a loopful of the culture is examined under the low power of the microscope. In the case of *L. tropica* the culture has a granular appearance, thick granular masses of flagellates standing out in a background of less dense growth, while in *L. donovani* the flagellates are almost uniformly diffused throughout the medium.

Clinical and epidemiological studies of the infections in man and other natural hosts

This is the principal method by which species and varieties of *Leishmania* have been separated.

The genus *Leishmania* was created by Ross for the parasite of Indian kala azar, *L. donovani*. When similar parasites were found in different types of infection new species of *Leishmania* were created for them. *L. tropica* (Wright, 1903) is now the generally accepted name for the parasite of oriental sore. Nicolle (1908) created the species *L. infantum* for the parasites of Mediterranean kala azar, owing to the peculiar restriction of the disease to young children. Further names were introduced after the discovery of American leishmaniasis, for the parasite of which Vianna (1911) first proposed the name *L. brasiliensis*. Laveran & Nattan-Larrier (1912) considered that the American parasite was closely allied to *L. tropica* and named it *L. tropica* var. *americana*, a view endorsed in several modern textbooks of tropical medicine, which do not discriminate between oriental sore and leishmaniasis americana. Velez (1913) proposed the name *L. peruviana* for parasites observed by him in cases of cutaneous leishmaniasis in Peru. Escomel (1913) found on three occasions forms with short flagella in lesions which he considered were clinically different from espundia, and proposed the name *L. americana* var. *flagellata*. On account of the supposed peculiarities of the lesions in human beings Brumpt (1913) created the species *L. nilotica* for the parasites found by Thomson & Balfour (1910) in certain non-ulcerating forms of cutaneous leishmaniasis. Dostrovsky (1934) used the name *L. recidiva* for parasites found by him in a lupus-like lesion of great chronicity, which had persisted for over six years, but Wenyon (1935), in a review, suggested that perhaps the author was naming the skin condition, and had intended writing 'leishmaniasis recidiva'. On rather similar grounds Castellani & Chalmers (1919) differentiated the parasites of Sudan kala azar as *L. donovani* var. *archibaldi*, and Chagas and his colleagues (Cunha & Chagas, 1937; Chagas *et al.* 1937) created the species *L. chagasi* for the parasites of South American kala azar, which they believed could also be differentiated from other *Leishmania* by animal inoculation and by immunological methods.

On account of their occurrence in a different host the parasites of canine leishmaniasis were named *L. tropica* var. *canina* by Yakimoff & Schokhor (1914) and *L. canis* by Nicolle (1908). More recent studies by Adler & Theodor (1930), Mills, MacHattie & Chadwick (1930), Sofieff & Schevtchenko (1934) and other workers all point to the conclusion that

there are strains of canine leishmaniasis indistinguishable from those of man. Cutaneous and visceral leishmaniasis in dogs is commonly, but not invariably, associated with the same type of infection in the human population. Evidence that there is a special type of leishmaniasis restricted to dogs has not yet been produced.

Animal inoculation

The results of animal inoculation are so variable that little information regarding the differentiation of *Leishmania* has been obtained by this method so far. The parasites of kala azar may in some instances give rise to purely cutaneous lesions in experimental animals (Row, 1912, 1913; Kirk, 1944), while *L. tropica* may produce either a localized lesion or a generalized infection in animals (Row, 1914). The latter parasite causes visceral leishmaniasis more frequently and much more intensively than *L. donovani* when inoculated into white mice (Adler, 1929), but Adler & Theodor (1930) found that different strains of this parasite vary in their ability to infect white mice. Some human and canine strains were found to be non-infective, others to produce visceral and cutaneous lesions after intra-peritoneal inoculation. With some strains of *L. tropica* the generalized infection in white mice is followed by secondary cutaneous infection round the ears, tail and scrotum (Parrot, 1928), recalling the muco-cutaneous leishmaniasis of South America. As a result of his extensive experience Adler (1947) concludes that 'the production of local lesions in the tails of mice is characteristic of strains of *L. tropica*'. This suggests that *L. tropica* is closely related to the parasites of *L. brasiliensis* which produce secondary muco-cutaneous lesions. On the other hand, the writer (Kirk, 1945) has on one occasion seen comparable lesions, including an oro-nasal one resembling espundia, in a monkey inoculated with parasites derived originally from the spleen of a case of Sudan kala azar.

Caminopetros (1936) found that the spermophil (*Citellus citellus*), although very susceptible to infection with *Leishmania donovani*, is refractory to visceral infection with strains of *L. tropica*. By inoculation of spermophils he concluded that certain forms of ulcerative cutaneous leishmaniasis in dogs in Greece were caused by secondary cutaneous localization following general infection with *L. donovani*, and not by *L. tropica* (Brumpt, 1936). Fuller & Geiman (1942) state that *L. brasiliensis* produces only local lesions in the Syrian hamster. This, if confirmed, constitutes an interesting biological difference between *L. brasiliensis* and *L. tropica*.

Hindle (1928, 1930) has shown that Chinese strains of *Leishmania* vary in their virulence for hamsters. He concluded also (1931) that hamsters were much less susceptible to infection with *L. infantum* than with Indian and Chinese strains of *L. donovani*. Hindle & Thomson (1928) studied the behaviour in hamsters of a strain of *L. infantum* isolated in Tunis by Nicolle. They found that visceral infection was succeeded by localized infection of the joints, testes and skin, and concluded that the behaviour of this strain in hamsters indicated that 'the Chinese and Tunisian strains of *Leishmania* may be sufficiently distinct to be regarded as different varieties, if not species'. Their failure to infect dogs with this Tunisian strain suggests, however, that it may have been rather unusual.

Adler (1938) and Cunha (1938) have now shown that the older view that the South American *L. chagasi* could be differentiated from other species of *Leishmania* by the fact that it would not infect laboratory animals was based on incomplete experimentation, since *L. chagasi*, like *L. donovani*, produces infection in hamsters, monkeys and dogs.

Some interesting results have been obtained by the inoculation of parasites from natural canine infections into human volunteers. Adler & Theodor (1930) produced purely cutaneous leishmaniasis in a human being by inoculating material from a naturally occurring oriental sore of a dog in Baghdad. A similar result was obtained in India by Sinton (1938), who produced oriental sore in an European by inoculation of material from an oriental sore of a dog. In this instance the strain was passed from the first human volunteer to a second, in whom also purely cutaneous leishmaniasis resulted. These observations support the view that as there are strains of *L. canis* in some places indistinguishable from *L. donovani*, so in other places *L. canis* may be identical with *L. tropica*. Russian workers (Hoare, 1944) found that parasites from the 'dry' and 'moist' types of oriental sore could be distinguished by inoculation into human volunteers who invariably developed sores corresponding in type with those from which the parasites had originally been derived. Kriukova (1941) found that the two parasites can also be differentiated by inoculation into gerbilles and white mice, which are refractory to infection with parasites from sores of the 'dry' type, whereas parasites from 'moist' sores from human and gerbille sources invariably produced infection.

Cross-immunity tests

In hamsters which recover from a leishmania infection there is generally immunity against a second infection with the same strain of parasite (Chung & Wang, 1939) but not against other types. Laveran (1917) records that a monkey which had acquired immunity against *L. donovani* was insusceptible when inoculated with *L. infantum*, but animals which had recovered from *L. tropica* infections were susceptible to inoculation with *L. donovani*.

Chung & Wang (1939) found also that hamsters recently cured of *L. donovani* infections were refractory to infection with *L. infantum*. Nicollè & Manceaux (1910) found that previous infection with *L. donovani* protected against oriental sore, but other workers, e.g. Parrot, Donatien & Lestoquard (1927) were unable to confirm this.

In the human subject a person who has recovered from kala azar, with or without treatment, apparently has almost complete immunity against a second infection with the same strain of parasite, but not against other *Leishmania* infections, such as oriental sore (Napier, 1946). The same is true of oriental sore; a person who has recovered from this infection may contract kala azar (Patton, 1922), but has almost complete immunity against a second infection with the same strain of parasite, a fact which has long been recognized by the people in some endemic centres, who made a practice of inoculating their children on some part of the body normally covered by clothes in order to avoid the disfiguring scar which might result from natural infection on the face. Immunity against one strain of oriental sore does not necessarily protect against other strains. Wenyon (1911*b*) noted that persons who have suffered from Aleppo boil, coming to Baghdad, have been known to contract the Baghdad lesion. Kojevnikov (1941, 1942) has shown that there is no cross-immunity between the 'moist' and 'dry' types of oriental sore in Middle Asia, and produced evidence of antigenic differences between these two types of *L. tropica* as great as those which can be demonstrated between *L. tropica* and *L. donovani*. From Hoare's (1944) review of the Russian work on cutaneous leishmaniasis one gains the impression that immunity in animals against the 'moist' type of infection is not lasting, and that second infections with the same type of parasite have been observed. In man the subject is complicated by the fact that development of immunity is a slow process, often requiring many months, and occurs only if the sores have been allowed to run their normal course and heal spontaneously, but not if they are prematurely terminated by treatment (Marzinowsky & Schourenkoff, 1924; Berberian, 1944).

Serological tests

Many workers have attempted the serological differentiation of *Leishmania*. The first to obtain clear-cut results was Noguchi (1924, 1926), who concluded that *L. tropica*, *L. donovani* and *L. brasiliensis* were antigenically distinct from each other, but that *L. infantum* was indistinguishable from *L. donovani*. Noguchi immunized rabbits by intravenous inoculation of cultures of the various *Leishmania* and found that the sera of immunized rabbits agglutinated the homologous organisms in dilutions of 1:10 or even 1:100. Similarly, the incorporation of immune serum in the culture medium inhibited the growth of the homologous organism, but not of the others. Noguchi's results were at once confirmed by Kligler (1925.)

Wagener & Koch (1926) found they could differentiate *Herpetomonas ctenocephali* from the *Leishmania* by serological methods, but the anti-leishmania sera affected equally the different strains of *Leishmania*. Ray (1929) produced immune sera by inoculation of rabbits with cultures of *L. donovani*, *L. tropica*, and *L. tropica* var. *americana* and found that such sera readily agglutinated the culture forms, but that in most cases they were not specific. He concluded that the separation of the parasites was not possible by serological means, including complement fixation.

Das Gupta (1930) concluded it was not possible to differentiate the organisms in post kala azar dermal leishmaniasis from *L. tropica* by serological methods. Nevertheless, all the evidence indicates that this condition is due to the parasites of kala azar, and is quite distinct from oriental sore.

Franchini & Pirani (1930) inoculated rabbits with cultures of *L. donovani*, *L. tropica*, *L. brasiliensis*, *L. tarentolae* and also various insect flagellates, and reported the production of immune sera which agglutinated only the homologous organism. Laurinsich (1931) found, as Noguchi had, that *L. infantum* was antigenically identical with *L. donovani*, but *L. tropica* and *L. brasiliensis* were distinct.

Zdrodowski & Woskressenski (1930, 1931) considered they could differentiate *Leishmania* by complement fixation, using antigens prepared from cultures. They had some difficulty with canine leishmaniasis, however, of which they concluded there were at least three types, two corresponding to the two human types, and a third which gave reactions with both parasites. Chodukin & Sofieff (1928, 1930) investigated the differentiation of *Leishmania* by immune sera, using agglutination, by effects on growth in culture, and also by the adhesion test. They concluded that the differentiation is not so easy as some observers have supposed, since each strain isolated may have features of its own.

In a paper reporting his own results and summarizing previous work, Row (1931) concluded that agglutination is a group reaction, and the agglutination test is of no value in differentiating various members of the genus *Leishmania*. Fonseca (1932, 1933) found, however, that immune sera prepared in rabbits against *L. brasiliensis* agglutinated the homologous organism and prevented its growth in culture, but had no effect in other types of *Leishmania*. After studying a large number of different strains of *Leishmania* of human and canine origin Chodukin, Sofieff & Kevorkov (1935) concluded that it was impossible to differentiate them by complement fixation, by Noguchi's method of

adding immune sera to the culture medium, or by agglutination tests, including the absorption of agglutinins by Castellani's method. They believe the reactions observed in serological tests are group reactions, unsuited for specific determination.

It was partly on the results of agglutination and absorption tests that Chagas and his colleagues (Cunha & Chagas, 1937; Chagas, *et al.* 1937) separated the parasite of South American kala azar as a distinct species, *L. chagasi*. Cunha (1938) demonstrated the presence of both somatic (thermolabile) and flagellar (thermostable) antigens in the culture forms, but after extensive experience he concludes that it is not possible to differentiate the species of *Leishmania* by serological methods, since all freshly isolated strains have a common antigen, and the secondary antigens which develop later in culture are non-specific.

Senekjie & Lewis (1945), using the lytic serum method of Noguchi with rabbit antisera to *L. donovani*, *L. tropica*, *L. brasiliensis* and *Trypanosoma cruzi*, report a specific action on the homologous organism. These workers (1944) have also demonstrated the presence of H and O antigens in the flagellates, and report agglutination results which are quite specific.

Messik (1928), Burowa (1928), Balachewa (1930, 1935), Chodukin & Sofieff (1928, 1930) and the writer (cf. Horgan, 1939) have attempted to use the Rieckenberg (or adhesion) phenomenon for the differentiation of *Leishmania*, with even less satisfactory results than those obtained by other serological methods.

The literature contains many other records in addition to those quoted which serve only to illustrate that there is much difference of opinion about the usefulness of serological tests for differentiating *Leishmania*. Although single investigators have often obtained clear-cut results, a general survey indicates that there may be marked differences in the behaviour of strains from the same type of infection. Workers with extensive experience, like Cunha or Chodukin and his colleagues, have found that the relatively clear results originally obtained with only a few strains become obscured when a large number of strains is studied.

Xeno-differentiation

Brumpt (1913) introduced the term xeno-diagnosis to indicate diagnosis by allowing arthropod vectors to feed on the patient and observing subsequently the development of specific parasites in the arthropod. Xeno-differentiation is a convenient word to indicate the differentiation of *Leishmania* according to the way in which they develop in different species of *Phlebotomus*.

The final demonstration that *P. argentipes* and *P. papatasii* can transmit kala azar and oriental sore respectively from man to man by the bite was preceded by a vast amount of entomological and epidemiological work which indicated that in nature the distribution of the *Leishmania* of man and dogs is as closely correlated (at least in the Old World) to the distribution of specific sandflies as malaria is to that of anopheline mosquitoes. When the Sergents, Parrot, Donatien & Beguet (1921) reported the experimental production of cutaneous leishmaniasis by rubbing into skin abrasions emulsions of sandflies collected from an endemic area of oriental sore, Sinton (1925) collected all the available information about the distribution of species of *Phlebotomus* in India, and found a correlation between the recorded distribution of *P. argentipes* and kala azar in India (cf. Knowles, Napier & Das Gupta, 1923). This observation led Knowles, Napier & Smith (1924) to undertake feeding experiments with *P. argentipes*. They fed specimens of *P. argentipes* on a kala azar patient and found that a heavy flagellate infection resulted in 25% of them; they also found that this degree of infection did not occur in other species of *Phlebotomus*. Other workers confirmed this observation and Shortt, Barraud & Craighead (1926) showed that in *P. argentipes* the infection passes forwards in the sandfly, eventually reaching the buccal cavity. This is in striking contrast with the course of development in true herpetomonads and leptomonads which are natural parasites of insects and tend to move backwards towards the rectum from which encysted leishmania forms are passed with the faeces.

Adler & Theodor (1926, 1928) found that *Leishmania tropica* behaved in *Phlebotomus papatasii* and *P. sergenti* in a similar way to that of *Leishmania donovani* in *Phlebotomus argentipes*. They found also that cultures of *Leishmania tropica* which had become non-infective for man regained their infectivity after passing through *Phlebotomus papatasii*. They tested also (1927, 1930) cultures of *Leishmania brasiliensis*, *L. infantum* and various flagellates from insects and plants in *Phlebotomus papatasii*. Some development occurred, but with the exception of one strain of *Leishmania infantum* (Paris strain) the infections showed no tendency to pass forward to the cardia and pharynx.

Similarly, Hindle (1928) found that the parasite of Chinese kala azar behaved in the same way in *Phlebotomus chinensis* as did that of Indian kala azar in *P. argentipes*. He found also (Hindle, 1931) that *Leishmania tropica*, *L. infantum* and a strain of *L. donovani* from India underwent a certain amount of development in *Phlebotomus chinensis*, but the infection did not pass forward to the cardia and pharynx. From those results Hindle concluded that the strains of *Leishmania* in different regions are biologically adapted to the local species of the vector and that, although there is a general capacity on the part of *Leishmania* to develop into the flagellate

stage in various species of *Phlebotomus*, it is only when a biological relationship exists between the two that development proceeds further and the flagellates invade the head and mouthparts.

A great deal of work has been carried out along similar lines which all tends to confirm the conclusions stated by Hindle. Briefly, it has been found in various endemic areas that the distribution of leishmaniasis is closely related to the distribution of a particular sandfly (different in each area) which is comparatively easy to infect with the local *Leishmania* and in which the local strains undergo the specific 'anterior' type of development described by Shortt, Barraud & Craighead (1926), the infection, moreover, persisting throughout the remainder of the life of the sandfly. In practice it is difficult to carry the matter beyond this point in every instance and show that the particular sandfly can, in fact, transmit the infection to man by the bite. The history of events in India during the last 20 years indicates that the difficulties are mainly technical, while the fact that transmission has now been successfully demonstrated seems (at least to the writer) to have eliminated the most cogent objection to the sandfly transmission hypothesis. It can now be accepted that the sandfly which is correlated with leishmaniasis in distribution and prevalence in any area, and in which the local strains undergo specific xenial development is, in fact, the local vector, to which, as Hindle suggested, the local strains are biologically adapted. This adaptation is not necessarily so highly specific as to exclude the possibility of anterior development occasionally occurring in other species of sandfly. Experimenting with a strain of Sudan leishmaniasis in *P. papatasii*, Adler (1947) has shown that this result can be produced by allowing the sandflies to take up comparatively enormous doses of infective material, such as are never likely to be encountered in nature.

The relation between local strains of *Leishmania* and local vectors is not the same as in malaria or yellow fever, both of which are now recognized as having different vectors in different places. The virus of yellow fever is transmitted equally well by many different mosquito vectors. If any particular species is found to be the local vector in a given area this is generally due to certain features in its bionomics which provide better opportunities for transmitting the infection from man to man than in the case of other equally efficient transmitters in the same area. There is no evidence of a specific adaptation of strains of the virus to local vectors which can be demonstrated in the laboratory.

Differentiation of *Leishmania* according to their specific xenial development in species of *Phlebotomus* requires the recognition of groups which are not separated from each other in the systems of classification already discussed. Thus, Indian and Chinese strains of *Leishmania donovani* can be differentiated on the grounds that the former are adapted to *Phlebotomus argentipes*, the latter to *P. chinensis*. The parasites of Sudan kala azar can probably be separated from both of those owing to their adaptation to different vectors such as *P. orientalis* (Kirk & Lewis, 1947), which does not occur in India or China. In the Mediterranean area different species of *Phlebotomus* have been shown to be vectors in different localities, e.g. *P. perniciosus* in Italy, *P. major* in Crete (Adler, Theodor & Witenberg, 1938). The latter species occurs also in India, but is apparently not regarded as a vector there. Similarly, strains of *Leishmania tropica* from Greece and Palestine differ in their behaviour in sandflies. The former produces a very low infection rate in *Phlebotomus papatasii* as compared with that in *P. sergenti*, whereas Palestinian forms appear to be biologically adapted to *P. papatasii*. Baghdad strains produce a high infection rate in *P. sergenti* and a low one in *P. papatasii*. Correlated with this the distribution of oriental sore in the district of Baghdad coincides with that of *P. sergenti* and not with that of *P. papatasii* (Adler & Theodor, 1931).

IV. DISCUSSION

In the system of classification accepted at present by both botanists and zoologists living organisms are separated from each other into species on *morphological* grounds. One disadvantage of this system is that aberrant forms or ecological variants which are often entirely phenotypic modifications may be described as species by the systematist. Also, it is now widely recognized in organisms which have been intensively studied that there exist groups which differ from each other physiologically or *biologically*, although they may be identical morphologically. In recent years there has been much discussion of these groups, which have at present no clearly defined taxonomic status.

The difficulty is not new. It was appreciated by bacteriologists who have attempted to evolve a scheme of terminology suited to the requirements of their work and at the same time consistent with the system of nomenclature used in other departments of biological science. Identification of species in bacteriology depends not only on the morphology of individual organisms and of growth in culture media, but also on physiological and biochemical characters and pathogenicity to animals under experimental conditions, while in antigenic analysis a technique of exquisite delicacy has been evolved for the differentiation of certain organisms which may be identical morphologically. It is interesting that a distinguished metazoan systematist like Mayr (1942) regards the definition of species according to antigenic constitution as 'basically a thoroughly

old-fashioned morphological species definition, based on the degree of difference'.

In the case of parasitic protozoa like the *Leishmania* which multiply by simple fission only it may be justifiable to use the methods of bacteriology for the differentiation of species which may be morphologically indistinguishable. On grounds of pathogenicity to experimental animals the *Leishmania* of lizards (*L. hemidactyli*, *L. agamae*, etc.) have been regarded as distinct from those occurring in man and dogs (Hindle, 1930). This separation is supported by the results of antigenic tests, and is apparently accepted by the systematists. In the *Leishmania* of man and dogs, however, these methods provide less reliable criteria for the differentiation of so-called species than those derived from clinical and epidemiological data, which represent the accumulated experience of years. The bacteriologist will not find this surprising. Even antigenic analysis which has been so illuminating in the *Salmonella* and other groups has not always been successful in bacteriology. The classical example is the failure of this method in the important genus *Mycobacterium* (tuberculosis).

In practice, many of the specific and varietal names mentioned earlier in this paper are now considered redundant and have, for one reason or another, fallen into disuse. Modern text-books on tropical medicine and parasitology dealing with human leishmaniasis now recognize two, or at the most, three species of *Leishmania*: *L. tropica*, *L. donovani*, and *L. brasiliensis*. These species are separated from each other, not on morphological differences between the parasites, but on the grounds of clinical, pathological and epidemiological differences in the infections produced by them in human beings, which have now been studied more or less continuously for over 40 years in various parts of the world in which they occur. But, although in general the classical forms of human leishmaniasis are distinct, intermediate and transitional forms of infection are sometimes seen (Kirk, 1942). Moreover, experimental studies of the parasites of kala azar and oriental sore have revealed remarkable differences between strains isolated from one type of infection, as, for example, cutaneous leishmaniasis in Russia.

Wenyon (1926) considered that the distribution of kala azar and oriental sore was against the view that *L. donovani* and *L. tropica* are identical, although he states that many arguments could undoubtedly be raised in support of their inclusion in one species. More recently Hoare (1943), after eliminating other species, concludes that the human and canine parasites described under the names *L. donovani* and *L. tropica* 'actually represent two biological races of the same species, for which the earlier name *L. donovani* should be reserved. One of these races produces a generalized visceral infection while the other gives rise to localized cutaneous or mucosal lesions.' However, in experimental animals this is not always the case. As regards naturally contracted leishmaniasis in the human host, the writer (Kirk, 1944) has shown that the infection tends to undergo a fairly specific course of evolution in which some of the cutaneous and mucosal lesions must be recognized as sequelae of a generalized visceral infection. This appears to be the case also with some of the cutaneous manifestations of canine leishmaniasis.

The taxonomic status of 'biological races' has not been defined. Mayr (1942) points out that many of the so-called biological races have recently been unmasked as geographical races, or ecophenotypes, or sibling species. He suggests that the term should be restricted to the host races of parasitic or semi-parasitic forms and of monophagous food specialists. The application of this rule in the *Leishmania* of man and dogs allows the recognition of biological races on the grounds of more or less well-defined host specificity, not in the vertebrate host, but in the insect vector. It is consistent also with conclusions derived from a study of the geographical distribution of *Leishmania* infections. There seems no need for any special system of nomenclature. The *Leishmania* of man and dogs may be regarded not as clearly defined species but as strains which are undergoing evolution in adaptation to the local vectors and other factors, varying in their virulence for human beings and animals, in susceptibility to chemotherapeutic agents and also in their tissue tropism. There is no indication that the recognition of biological races on the grounds of adaptation to specific vectors is inconsistent with the experience of those who have studied leishmaniasis clinically. In fact there is some indication that biological races of *Leishmania* thus defined could be grouped into a few higher categories corresponding roughly with the *L. donovani*, *L. tropica* and *L. brasiliensis* of medical text-books. It is essential that the importance of the clinical records be recognized since the infections produced by these parasites have been under practically continuous observation for 50 years, a period which covers the lives of innumerable generations in a unicellular organism like *Leishmania*. Clinical differences in the types of infection occurring in different areas have persisted during this period, so we are justified in concluding that they are the result of biological differences in the parasites which are hereditarily stable.

Differentiation of *Leishmania* by this method would probably permit the clear separation of the American forms as a distinct group in view of the great differences between Neotropical sandflies and those of the Old World. If it is considered desirable to maintain *L. donovani* and *L. tropica* as separate species then there is also some justification for regarding the *Leishmania* of the New World as a distinct group from those of the Old World, in which

case the law of priority indicates the use of Vianna's name *L. brasiliensis* for the forms causing American cutaneous and muco-cutaneous leishmaniasis. The position is still uncertain as regards South American visceral leishmaniasis.

V. NOMENCLATURE OF *LEISHMANIA*

As far as the writer is aware the complete synonymy of the species of *Leishmania* of man has never been published. Hoare (1938) published a list of the synonyms of *L. donovani* and *L. tropica* which omits the *Leishmania* of the New World and many of the names mentioned in this paper. The list below contains all the names the writer has been able to trace which have been given to the parasites of human leishmaniasis. A strict systematist would regard it as merely a list of the synonyms of one species, *L. donovani*, but the names have been divided into three groups according as they were assigned to parasites causing oriental sore, kala azar or American cutaneous and muco-cutaneous leishmaniasis. No attempt is made to express any other differentiation suggested in this paper.

(A) Strains causing oriental sore:

Sporozoa furunculosa Firth, 1891
Helcosoma tropicum Wright, 1903
Leishmania furunculosa (Firth, 1891) Blanchard, 1904
Ovoplasma orientale Marzinowsky & Bogrow, 1904
Leishmania tropica (Wright, 1903) Lühe, 1906
L. wrighti Nicolle, 1908
L. cunninghami Carter, 1909
Crithidium cunninghami Carter, 1909
Herpetomonas tropica (Wright, 1903) Patton, 1909
Leishmania nilotica Brumpt, 1913
Plasmosoma jerichaense Huntemuller, 1914
Leishmania tropica var. *canina* Yakimoff & Schokhor, 1914
L. tropica var. *major* Yakimoff, 1915
L. tropica var. *minor* Yakimoff, 1915
Herpetomonas furunculosa (Firth, 1891) Patton, 1922
Leishmania recidiva Dostrovsky, 1934.

(B) Strains causing kala azar:

Piroplasma donovani Laveran & Mesnil, 1903
Leishmania donovani (Laveran & Mesnil, 1903) Ross, 1903
Herpetomonas donovani (Laveran & Mesnil, 1903) Mesnil, 1906 (*nec* Patton, 1908)
Leishmania infantum Nicolle, 1908
L. canis Nicolle, 1908
Leptomonas donovani (Laveran & Mesnil, 1903) Mesnil, 1909
Leishmania donovani var. *archibaldi* Castellani & Chalmers, 1919
L. chagasi Cunha & Chagas, 1937.

(C) Strains causing American cutaneous and muco-cutaneous leishmaniasis:

Leishmania brasiliensis Vianna, 1911
L. tropica var. *americana* Laveran & Nattan-Larrier, 1912
L. peruviana Velez, 1913
L. americana var. *flagellata* Escomel, 1913.

SUMMARY

1. The nomenclature and synonymy of the *Leishmania* of man and of the dog is reviewed.

2. Many of the older names have been discarded and medical writers now recognize only two, or at the most three, species of *Leishmania* of man and dogs.

3. These species are indistinguishable morphologically. Attempts to differentiate them by the techniques of bacteriology have given conflicting results and there is no general agreement that they can be so differentiated.

4. It has been suggested that separation of species of *Leishmania* is not justified under the present rules of systematics and that all forms of human and canine leishmaniasis must be regarded as the result of infection by a single species of parasite.

5. This suggestion is not entirely acceptable in view of contributions to the subject by medical workers who have studied the results of infection in the human subject intensively and continuously for half a century.

6. Evidence is produced that the different types of human leishmaniasis recognized clinically can be correlated with biological differences in the causal parasites.

7. In other organisms which have been intensively studied the existence of groups which differ from each other in biological characters only is widely recognized in modern systematics, but there is no general agreement about nomenclature for the definition of such groups.

8. Reasons, not inconsistent with modern studies in systematics, are given for the recognition of at least three groups in the *Leishmania* of man, corresponding with the *L. donovani*, *L. tropica* and *L. brasiliensis* of medical text-books. Definition of the taxonomic status of such groups will depend on the further progress of systematics.

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THE ECOLOGY OF IMMATURE PHASES OF TRICHOSTRONGYLE NEMATODES

III. LARVAL POPULATIONS ON HILL PASTURES

By H. D. CROFTON, B.Sc., Ph.D., *Department of Zoology, University of Bristol*

(With 3 Figures in the Text)

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I. INTRODUCTION

This work is primarily a study of some of the factors which influence the fluctuations in populations of trichostrongyle larvae on hill pastures.

A previous paper (Crofton, 1948*b*) deals with the activity and survival of the immature stages of rabbit trichostrongyle, *Trichostrongylus retortaeformis*. Although the detailed conclusions cannot be assumed to apply to the immature stages of trichostrongylid worms in sheep, the general principles have some application to the present problem, for factors which affect the activity and survival of larvae play an important part in controlling the fluctuations of populations. Ecological are not the only factors concerned, however, and in studying populations the continual renewal of the infestation must be considered. This renewal of infestation is dependent upon the density of the host population, the habits of the host animals and changes in the hosts' resistance to infestation.

All of these factors may be studied separately, but their combined effect is not easily predictable. Direct determination of fluctuations in population gives a measure of the integrated effect of all of these factors, but again there is the disadvantage that it is impossible to separate the component effects. Most of the work in this field has been directed to the study of individual factors, and little attention has been paid to the direct approach. The latter method, that of sampling pastures at frequent intervals, has been adopted in this work, special attention being paid to the distribution of the larvae, and the grazing habits of sheep.

II. METHODS

The number of larvae on a pasture was estimated by the method described by Taylor (1939). A few modifications were introduced to give greater accuracy: large funnels with wide stems were used instead of buckets; large numbers of small samples were taken, and these were not pooled as suggested in the original description. Methods of sampling and separation of larvae will be discussed in a later paper.

III. THE EFFECTS OF DIFFERENT METHODS OF SHEEP-HUSBANDRY ON PASTURE CONTAMINATION

(*a*) *Shepherding*

Methods of flock management vary in different parts of the country. In many districts hill sheep are left undisturbed on the hills, except when sheep are gathered for clipping, dipping and sorting. These flocks are inspected regularly, sometimes daily, but more usually only once or twice a week. The frequency of inspection varies with the type of terrain and with the season. This will be referred to as the 'fixed-flock' type of management.

In other districts, chiefly in the Cheviot Hills of Northumberland, the sheep are inspected twice daily and the flock moved at each inspection. These sheep, which pass the night at the top of the hill, are moved downwards in the morning and continue moving as they graze, reaching the lowest part of the pasture about midday. The shepherd then turns the sheep, and they move upwards during the afternoon, reaching the summit by nightfall.

In the 'fixed flock', the individual sheep keep to a fairly limited portion of the pasture. This portion may be several acres in area, and several sheep may graze it, the grazing areas of individuals overlapping. In the 'moving flock', however, the individual sheep do not maintain a fixed grazing area, but may maintain a fairly constant position in relation to the rest of the flock.

The relative merits of these two types of management cannot be discussed here, the differences being mainly the result of differences in type of pasture. The 'moving-flock' method, however, seems to have one advantage which is important in controlling parasitic infestation.

Observations made at night showed that sheep pass most of the hours of darkness during summer lying on the ground. Some grazing occurs, but much less than during the day. During observations on a bright, moonlight night, it was noted that less than 10 % of the sheep visible were grazing at any one time. During the day, except during the hottest period, more than 60 % were grazing simultaneously. Thus, in the 'moving flock', little grazing occurs at the summit of the hill.

Evidence as to the rate of defaecation is difficult to acquire in the field, but if it is assumed that defaecation is not limited to times of grazing, then the greatest concentration might be expected to be found on the hill top in the 'moving flock'. To verify this, examinations were made on two different types of hill pastures.

A wooden frame, 12 in. square, was used in the same way as in making a botanical survey. The frame was thrown at random and the faeces lying within the frame collected and weighed. On the 'fixed-flock' hill it was found that the distribution was not random, and the results could not be analysed. On the 'moving-flock' hill similar results were obtained on the slopes, but a rough comparison showed that the concentration at the summit was at least twenty times as great as on any other portion of the pasture.

Since sheep graze less at night than during the day, the 'moving-flock' type of management reduces the chances of infestation by reducing the amount of faeces on the grazed portions of the pasture.

(*b*) *Uneven grazing*

While the 'moving-flock' type of management is said to produce a more evenly grazed pasture than the 'fixed-flock' type, even the best hill farms have a high proportion of ground which is never grazed by sheep. On these farms, the proportion may be as high as 20 %, while the area grazed on poor farms may be less than 50 % of the total acreage.

Thus, while the theoretical stocking may be one sheep for every three acres, there may be less than one acre of grazed ground for each sheep.

The grazed portions are usually small patches partially separated from one another by islands of coarse vegetation. Sheep, being selective grazers, tend to accentuate this deterioration of the pastures. (Many agriculturists believe that this deterioration has occurred more rapidly in recent years because of changed methods of management. The increase in the proportion of young sheep in a flock by the earlier sale of wethers has, they assert, contributed to uneven grazing, and also to an increase in the incidence of parasitic disease in hill sheep.)

Visual examination of the slopes of a hill pasture suggests that most of the faecal pellets are distributed over the grazed portions. To determine the effect of uneven grazing on parasite populations samples were taken from various portions of a pasture, the portions selected being classified as 'rough' or 'short', the 'rough' being assumed to be less grazed than the 'short' areas (see Table 1).

Table 1

	No. of larvae per lb. of grass			
Pasture ...	A	B	C	D
Random samples	20	145	165	110
'Short' areas	400	300	480	520
'Rough' areas	0+	10	15	10

0+ = less than 10 larvae per lb.

There was a very great difference in numbers of larvae on grazed and ungrazed portions, and random sampling of the whole pasture did not give a satisfactory measure of the population available to the hosts. However, the difference between the number of larvae on well-grazed and rough areas is increased by estimating the number of larvae on a grass weight basis, the estimates being weighted in favour of large catches from short grasses. The distribution of the larvae can be determined more accurately by estimating the number of larvae per unit area of pasture. In practice this is difficult, but it is possible to apply a correction figure to remove weighting of the estimate.

One hundred samples were taken from each of the two areas of each pasture, rough and short. The samples consisted of all grass within a circular frame 1 in. in diameter. The samples were weighed, the ratio, *weight of grass from rough areas : weight of grass from grazed areas* being determined. Correction figures for each pasture were thus obtained, short grass lengths being given unit value for each pasture. Table 2 shows corrected estimates for pastures B, C and D.

Clearly, the number of larvae per unit area is greater on the grazed than on the ungrazed areas, even though the method of correction has given

too much weight to the 'rough areas' estimate in Table 2. This over-weighting has occurred because no correction has been applied to compensate for differences in vertical distribution of the larvae. (Crofton (1948*a*) showed that the vertical distribution was correlated with grass length. Kauzal (1941) showed that the density of the herbage affected the number of larvae migrating.)

IV. FLUCTUATIONS IN POPULATIONS ON PASTURES

(*a*) *Fluctuations throughout the day*

Air temperatures, humidities, light and rates of evaporation vary with the time of day, and these factors affect the vertical distribution of larvae. Changes in these factors are not necessarily closely

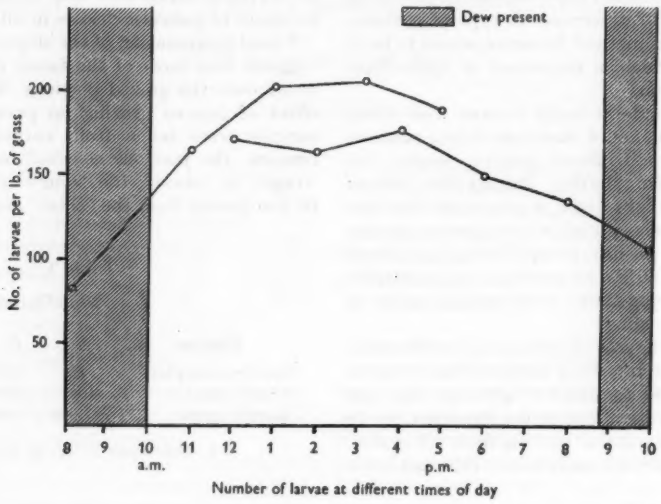

Fig. 1

Table 2

Pasture ...	No. of larvae B	C	D
Correction factor	12·5	5·5	17
'Short' areas	300	480	520
'Rough' areas	10	15	10
'Corrected' 'rough' areas	125	83	170

While the application of correction factors is important in determining where the highest concentration of larvae occurs, the number of larvae per lb. of grass is the best measure of the chances of infestation of sheep, and it has been shown that the greatest concentration of infective larvae occurs on well-grazed portions of pastures. Thus, uneven grazing produces localized concentrations of larvae, so that on hill farms the apparent low stocking is not necessarily a safeguard against outbreaks of parasitic disease. Further, estimates of infestation of a hill pasture will be misleading when Taylor's original method of sampling is adopted.

reproduced in the herbage, however (Crofton, 1948*a*), and the variation in the number of larvae on grass blades throughout the day was not known.

Because such variation might affect estimates of population the following observations were made.

A pasture was inspected, and on the basis of distribution of faecal pellets and conditions of herbage, an evenly grazed area, approximately 50 yards square, was chosen. Samples were taken from this portion of the pasture on 5 June at 8 a.m., 11 a.m., 1 p.m., 3 p.m. and 5 p.m. G.M.T. On 8 June samples were taken at 12 noon, 2 p.m., 4 p.m., 6 p.m., 8 p.m. and 10 p.m. G.M.T. The sampling method was identical in each case, the grass being cut with scissors near the base of the blades, care being taken to include as little 'mat' as possible.

Fig. 1 summarizes the results, these being similar to those obtained when the observations were repeated in July and August. Readings taken between 12 noon and 5 p.m. remained constant within the limits of sampling error. The difference between the 7 a.m. and the 11 a.m. readings (80 larvae per lb.) was undoubtedly a real one and not

due to the technique employed. In the morning and evening, when temperatures were low, fewer larvae were found on the grass blades than in the afternoon. The lowest number of larvae was recovered when dew was on the grass (see Fig. 1).

Thus, in estimating pasture populations, samples taken between 12 noon and 5 p.m. will give maximal estimates, and within these limits the time of sampling is immaterial. Before noon, and after 5 p.m., however, there may be considerable reduction in 'catch', the degree of reduction presumably depending upon weather conditions, both past and present, and upon the density of herbage. Also, an important point emerges: when the grass is wet with dew, the number of larvae on the grass blades is low. One recommendation for the control of parasitic infestation is that young stock should be prevented from grazing when dew is formed, and this is based upon the observation that moisture is necessary for the migration of larvae. Although moisture is undoubtedly necessary, direct observation shows that the recommendation is unnecessary and misleading because the theory underlying it is at fault, since it does not recognize that moisture is only one of the factors operating. Little or no migration of trichostrongyle larvae occurs when temperatures fall below 50° F. (Buckley (1940) on larvae of *Haemonchus contortus*, Rogers (1940) on mixed cultures of trichostrongyle larvae, Crofton (1948*b*) on *Trichostrongylus retortaeformis*).

Two conditions are necessary for the formation of dew on grass. The atmospheric humidity must be high, the grass temperature must be lower than the air temperature. Examination of temperature records taken on hill farms in Northumberland showed that temperatures at 7 a.m. G.M.T. during the months of June, July and August were never above 52° F., and on 57 % of days they were below 48° F. Thus, when dew was formed, the grass temperature would be below 52° F., and frequently below 48° F. Little or no migration would occur at these times. The low catches at 7 a.m. confirm this.

Fig. 2

(*b*) *Weekly fluctuations*

The pasture chosen for this study was a hill grazing of approximately 550 acres carrying Blackface ewes and lambs (lambing time at end of April). Occasional deaths from parasitic disease in lambs had occurred in previous years. Post-mortem examination had shown that *Ostertagia circumcincta* and *Trichostrongylus axei* were the most common species in the dead lambs on this farm. Anthelmintic treatment was not given regularly, and in 1945, when these

observations were made, only one treatment was given, each sheep receiving 15 g. of phenothiazine during the middle of October. An evenly grazed area, approximately 100 yards square, was chosen because of the relative uniformity of its herbage. Weekly samples were taken from this area during the period June–October 1945. Samples were taken on four occasions during the period 1 March–1 June 1945, and another four samples were taken during November and December 1945. Seven samples were taken during March–June 1946. Samples were always taken between 2 and 4 p.m. G.M.T. Fig. 2 summarizes the results, catches being recorded as numbers of larvae per lb.

In the warmer period June–October, when maximum temperatures were above 50° F., more than 200 larvae per lb. of grass were recovered on each occasion. When maximum temperatures fell below 50° F. (November and December 1945 and March–May 1946) less than 50 larvae per lb. were recovered. The greatest number of larvae occurred at the end of August, whereas the warmest period was during the first weeks of July.

De Blieck & Baudet (1926), Parnell (1934) and Britton (1938) say that the critical temperature for hatching of horse strongyle eggs is about 50° F. Lucker (1941) found that horse strongyle eggs hatched when maximum temperatures were below 46° F. Veglia (1915) found that eggs of *Haemonchus contortus* hatched at temperatures as low as 46·4° F., whereas Shorb (1944) observed that the larvae did not develop to the third stage below 55° F. Crofton (1948*b*) showed that eggs of *Trichostrongylus retortaeformis* did not hatch when maximum temperatures were below 50° F. Low catches recorded in the weekly observations on the pasture may be ascribed to the low temperatures preventing hatching and development. Since, however, some larvae were recovered in November and May, low temperatures must have had two effects. First, low temperatures prevented or retarded hatching and development; secondly, low temperatures reduced the rate of migration of the infective larvae, which had either survived from the warm period, or which had recently hatched (see Rogers (1940) on trichostrongyle larvae, Buckley (1940) on *Haemonchus contortus*, Crofton (1948*b*) on *Trichostrongylus retortaeformis*).

The number of larvae recovered in the warm periods does not show any correlation with temperature, however. The rate of hatching, development and migration must have been greatest during July (average weekly maximum 67° F., average weekly minimum 49° F.). Moisture conditions being suitable, this period should have shown maximal catches from a static population. Catches were greatest in August, however, when temperatures were lower than in July, so that the increase in numbers must have been due to a change in the total population attributable to an increase in the worm burden of the sheep. The time difference is too great to suggest that the population maximum in August is due to a lag imposed by the time necessary for development from egg to infective larva (see Threlkeld, 1934).

V. EFFECT OF CHANGES IN STOCKING

On lowland farms many variations of a system of rotational grazing are employed, but on the hill farms this is not possible. Usually, however, flocks are moved once or twice a year. During winter a portion of the flock is moved to pastures on lower ground, and often all the hoggs are wintered on a lowland farm. In summer, the whole flock, or some unit of it, may be moved. Usually the sheep are moved from a pasture consisting mainly of 'bent' (white-land) to a grazing where 'ling' predominates (black-land). Various reasons are given by farmers for this 'heather-change', but undoubtedly it gives no direct or immediate benefit, for the farmer is usually satisfied if weight is maintained. Most farmers assert that the sheep are more healthy and thrive more rapidly after their return, but it is difficult to assess the accuracy of farmers' claims as to the value of the 'heather-change', and weighing experiments cannot be adequately controlled. It was thought, however, that this practice might have some bearing on parasitic control, and that improvement in the health of the stock might be due to a reduction in pasture infestation. Taylor (1938) suggested that resting a pasture for a short period greatly reduced the number of infective larvae. Crofton (1948*b*) showed that the reduction in numbers of infective larvae was greatest in the summer months. To determine the effect of removal of stock, samples were taken from an evenly grazed portion of pasture from which the sheep were to be removed. Sampling was commenced in June and continued weekly until the end of October. The sheep were removed on 24 July, and returned 3 weeks later.

Fig. 3 summarizes the results, catches being recorded as the number of larvae per lb. of herbage. No attempt has been made to correct the estimates for change in length of grass, potential rate of infestation of sheep being more important than actual numbers in this case.

The number of larvae recovered increased from June to August, the catch increasing even after the stock had been removed. Twelve days after the removal of the sheep the number of larvae recovered was slightly less than on 24 July. One week later the number recovered was only 45 % of the 24 July catch. Five days after the return of the stock there was a further reduction and less than 20 % were recovered. Subsequent readings showed an increase,

but the highest catch was less than 35% of the previous maximum. In September the catches were progressively reduced, and in the first week of October only 40 larvae per lb. were recorded.

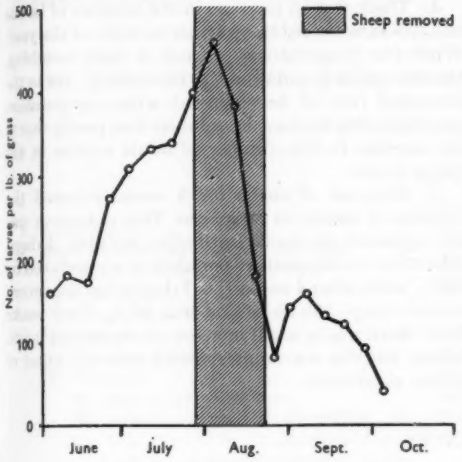

Fig. 3

VI. DISCUSSION

The results of the investigation may be stated briefly as follows: (1) Controlled grazing reduces the number of parasites on the grazed portions of a pasture by limiting the extent of faecal contamination. (2) Uneven grazing produces local concentrations of infective larvae on grazed portions of the pasture. (3) There is a fluctuation in the number of larvae on grass blades throughout the day, the lowest number being found when dew is formed, the highest number occurring between 12 noon and 5 p.m. G.M.T. During the latter period the numbers remain fairly constant. (4) There is a gradual increase in the number of larvae on a pasture during summer, the maximum number occurring in August. This peak does not coincide with the period of maximum temperature. (5) Resting a pasture for 3 weeks reduces the population by as much as 90%.

The system of controlled grazing, whereby the sheep move over the whole pasture during the day and remain on the hill-top at night, is one of the important ways in which the rate of increase of parasite populations is reduced. It ensures that a proportion of faeces passed by the sheep does not contaminate the well-grazed portions of the pasture. This will be effective in controlling the infestation of the sheep by trichostrongyles, but it must be realized that it increases the opportunity of other forms (e.g. *Bunostomum* sp.) which gain entry into the host by boring through the skin, because the sheep lie on this portion of the pasture at night.

Controlled grazing, however, has not prevented deterioration of pastures, and where deterioration has occurred there are large portions of the pasture which are never grazed. This has been shown to produce local concentrations of infective larvae on the grazed portions. Thus, improvement of pastures, and subsequent increased stocking may not necessarily increase the incidence of parasitic infestation, for the production of a more uniform sward will tend to distribute the larvae over the whole pasture and reduce the local concentrations. This is applicable where improvement is measured by the extent to which the whole pasture can be grazed by the sheep. A great increase in the nutritional value of the herbage may introduce problems of overcrowding, but the present status of hill farming is such that a policy of improvement by producing a more uniform grazing is more practical than one which advocates raising the nutritional value of all the herbage. In this case there seems to be little danger of an increase in parasitic infestation.

The observations on the fluctuations of numbers of larvae throughout the day are important in studying times and methods of pasture sampling, but they can have little bearing on parasite control on hill farms. Hill sheep and cattle remain on a pasture overnight, and there can be no question of limiting periods of grazing. The small number of larvae on grass blades when dew is formed may be of considerable interest on lowland farms, but this will be discussed in a later paper. It is obvious, however, that dangers of parasitic infestation are reduced rather than increased when grass is wet with dew.

Weekly examinations showed that the number of larvae on a pasture increased in the warmer months, reaching a maximum in August. Because the time of this maximum did not coincide with the period of highest temperature it can be assumed that the increase in numbers is not entirely due to increased rate of development, but that it is partly due to an increase in total population. This population increase must, however, be a reflexion of the increase in the number of adult parasites, so that the host is still susceptible at this time. Methods of control, therefore, should be introduced during, or before, August.

The great reduction in the number of infective larvae on a pasture following the removal of the sheep suggests that this is a valuable method of control. The 'heather-change' should be more widely adopted, therefore, and not restricted to 'white' land farms. Even though the pasture be rested for only 2 weeks the decrease in the number of infective larvae may be considerable. Ideally, however, the period should be longer, for the results show that after 12 days there was no apparent reduction, although the numbers recovered 1 week later were much lower.

This investigation, while not an exhaustive one, gives a basis for the design of methods of control of trichostrongyle parasites infesting hill sheep. Specific recommendations cannot be given, because the conditions from farm to farm vary considerably, and each farm must be considered individually.

VII. SUMMARY

Observations on the larvae of sheep trichostrongyles on hill pastures showed:

1. A reduction in larval populations occurred when the 'moving-flock' type management was used. The large proportion of eggs passed in faeces is deposited on a part of the pasture where little or no grazing occurs.

2. Local concentrations of larvae occurred on the grazed portions of a pasture when grazing was uneven. Thus, the apparent low rate of stocking on hill pastures does not give a true picture of the potential dangers of parasitic disease. Estimates of pasture contamination by Taylor's method of sampling were deceptively low.

3. The number of larvae on grass blades was lowest when the grass was wet with dew. Sampling of pastures between 12 noon and 5 p.m G.M.T. gave maximal and most constant estimates.

4. There was an increase in the number of larvae on a pasture during the warmer months of the year. While low temperatures prevent or delay hatching, the increase in population was not entirely due to the increased rate of development when temperature was high. The increase in numbers was partly due to an increase in the number of adult worms in the sheep hosts.

5. Removal of sheep for 3 weeks reduced the number of larvae on a pasture. This reduction was not apparent, however, until after the first 12 days. After 3 weeks the pasture population was reduced by 55 %, while after 4 weeks (i.e. 7 days after the return of the sheep) the reduction was 90 %. Two weeks later there was a small increase in the larval population, but this was again reduced with the onset of colder conditions.

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THE KINETICS OF THE PENETRATION OF SOME REPRESENTATIVE ANTHELMINTICS AND RELATED COMPOUNDS INTO *ASCARIS LUMBRICOIDES* VAR. *SUIS*

By A. R. TRIM, B.A., Ph.D., *Biochemical Laboratory, University of Cambridge*

(With 9 Figures in the Text)

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I. INTRODUCTION

In an earlier paper, Trim (1944), it has been suggested that, provided a toxic substance is able to reach the site of a parasite in its host, two major factors will control its antiparasitic activity:

(1) Its power of penetration into the parasite.

(2) The influence of the environment on the rate of penetration.

Further data are presented here to demonstrate the contribution of these factors to the anthelmintic action *in vitro* of (*a*) certain phenolic compounds; these are resorcinol, propyl resorcinol, butyl resorcinol, hexyl resorcinol and heptyl resorcinol, which are members of the homologous series of 4-*n*-alkyl resorcinols; and thymol, carvacrol and α-naphthol; (*b*) the alkaloid nicotine; and (*c*) the chlorinated hydrocarbon chloroform.

II. EXPERIMENTAL MATERIAL AND METHODS

For all experiments fresh specimens of *Ascaris lumbricoides* var. *suis* were kept under the conditions and obtained from the sources described by Baldwin (1943). Except where other conditions are mentioned the experiments were conducted at pH 6·8 in Baldwin's isotonic saline medium at 37° C.

(1) *Phenolic substances*

The rates of penetration of phenolic substances were compared by the technique described by Trim (1944) for the study of the penetration of hexyl resorcinol into *Ascaris*. This technique estimates, by means of a colorimetric method, the rate of removal of the drug from the medium by the worm. The method is based on the well-known azo-dye reaction of phenols, and was found to be applicable to all the substances studied. The colours produced by these compounds are shown in Table 1.

Table 1. *Colour of azo-dye in alkaline solution*

Phenolic substance	Colour of azo-dye formed with *p*-amino-benzoic acid
Resorcinol	Red
Propyl resorcinol	Orange-red
Butyl resorcinol	Orange-red
Hexyl resorcinol	Orange-red
Heptyl resorcinol	Orange-red
Thymol	Orange
Carvacrol	Orange
α-Naphthol	Red

(2) *l-Nicotine*

Nicotine was purified for use by distillation under reduced pressure (b.pt. at 16 mm. 122–123°, refractive index $1{\cdot}5230^{20}$), and estimated by the colorimetric method described by Trim (1948). Very small amounts of nicotine penetrate into *Ascaris* under the conditions of these experiments, and changes of its concentration in the medium cannot be detected. Consequently, it was necessary to determine the amounts of nicotine in the treated

worm by subjecting it to the distillation procedure for the recovery of nicotine (Trim, 1948).

Nicotine contains a weakly basic as well as a strongly basic group (p. 283), so that it was important to buffer all solutions used in penetration experiments to ensure that the drug remained in the same state of electrolytic dissociation throughout the experiment. The buffer counteracted the tendency of the worms to increase the hydrogen ion concentration in the medium by the excretion of CO_2 and organic acids. The ratio of the volume of medium to the volume of worm was large (*c.* 25 : 1).

(3) *Chloroform*

Chloroform was estimated by the micro-colorimetric method of Cole (1926). This is based on the reaction of chloroform with pyridine in the presence of concentrated soda which produces a magenta-coloured derivative of unknown constitution. Some minor modifications were introduced. 1 ml. samples of the chloroform solution to be estimated were put into small Kjeldahl combustion flasks each containing 2 ml. 40 % NaOH and 2 ml. pyridine purified by fractional distillation. Cooling fingers were inserted and the tubes heated on a boiling water-bath for 2 min. The colour developed in the pyridine layer. The tubes were cooled and the solution made up to volume with distilled water in 15 or 25 ml. volumetric flasks. The addition of a small volume of water gave a clear homogeneous solution. When a green filter (Chance's Filter O, Gr. 1) was used, Beer's law applied over a range of concentrations up to 1 mg. of chloroform/ml. in the original solution. The error was ± 2 %. Whenever it was necessary, chloroform was recovered from worm tissues by the method of extraction described by Cole (1926).

The volatilization of chloroform from aqueous solutions caused difficulties in the experimental work. Losses by volatilization were reduced to a constant minimum by the method described below and results of sufficient accuracy for comparative studies were obtained. The experiments were made in glass-stoppered, graduated cylinders of total volume *c.* 130 ml. Known weights of worms of uniform size and in batches of six were inserted at room temperature, a few glass beads were added for mixing purposes, chloroform solution, at room temperature, was measured in, up to the stopper, and the cylinders were immediately immersed in a thermostat at 37°. The temperature of the chloroform solution rose to 37° within 5 min. The fluid in the cylinder was kept mixed by frequent inversion and shaking. At intervals, 1 ml. samples of the medium were pipetted out and transferred to tubes prepared for the chloroform estimation. Samples of standard solutions were taken at each interval from identical cylinders containing the chloroform solution but no worms. Losses of chloroform by evaporation were determined in this way. The samples removed were replaced by the same volume of the original chloroform solution. The absolute losses due to volatilization under these conditions amounted to less than 5 % after incubation for 1 hr. at 37°. All cylinders were tested for losses before use.

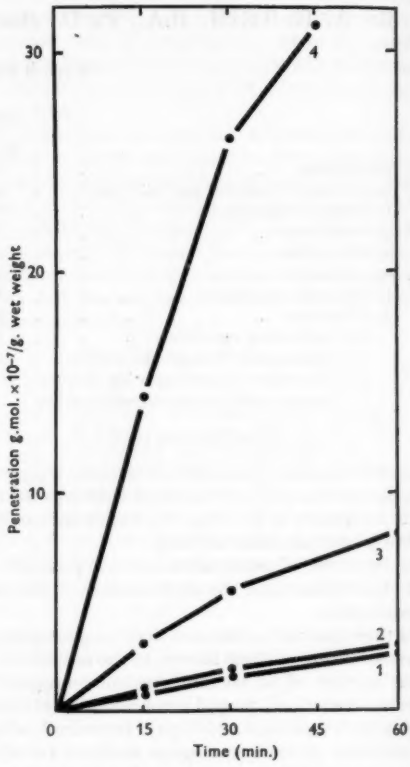


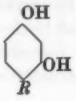
Fig. 1. Diagram showing the rates of penetration of resorcinols into *Ascaris* from 0·00167 M solutions. (1) Resorcinol, (2) 4-*n*-propyl resorcinol, (3) 4-*n*-butyl resorcinol, (4) 4-*n*-hexyl resorcinol.

III. EXPERIMENTAL

(1) *Phenolic substances*

Fig. 1 shows the relative rates of penetration of some members of the homologous series of 4-*n*-alkyl resorcinols from dilute equimolar solutions (0·00167 M). The diagram is plotted from the results of four determinations of drug uptake by six worms in 100 ml. of medium in each case. Fig. 2 shows the relationship between the logarithm of the initial rate of penetration and the number of carbon atoms

in the alkyl chain R of the resorcinol derivative (1).

(1) 

Because of its low solubility in water heptyl resorcinol could not be made up to a 0·00167 M solution. However, when the experiments were repeated with 0·000172 M solutions of the resorcinols heptyl resorcinol was sufficiently soluble to give a true solution. It was then found that it penetrates into *Ascaris* at about 2·5 times the rate of hexyl resorcinol of the same molarity, and fits the logarithmic relationship.

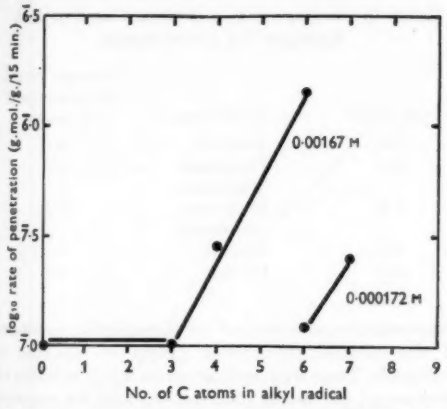
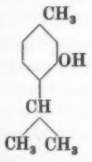
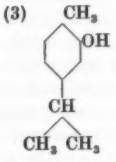


Fig. 2. Diagram showing the relation between the initial rates of penetration of resorcinols into *Ascaris*, and the number of carbon atoms in the alkyl radical.

Fig. 3 shows the rate of penetration of the two isomers thymol (2), carvacrol (3) and α-naphthol from 0·00167 M solutions. The rate of penetration of 0·00167 M *n*-butyl resorcinol has been inserted for comparison.

(2) 

(3) 

(2) *l*-Nicotine

(a) *Individual variations*

As in the case of the other substances investigated, large differences in the rate of penetration of nicotine into individual worms were found, which could not be accounted for in terms of the differences in the surface area of different individuals. For example, in a batch of twenty-two worms of approximately equal proportions, immersed in the same nicotine solution, the greatest deviation from the mean value of nicotine uptake/g. wet weight of worm was 75 %. The mean deviation was 24 %. This variation was allowed for in the planning of experiments.

(b) *Penetration through the cuticle*

Under the *in vitro* conditions employed the drug penetrates almost exclusively through the cuticle of the worm. This was demonstrated by comparing the amounts of nicotine penetrating into a batch of worms ligatured at the head and anus and the amount penetrating into a similar batch of unligatured worms. An example is given in Table 2.

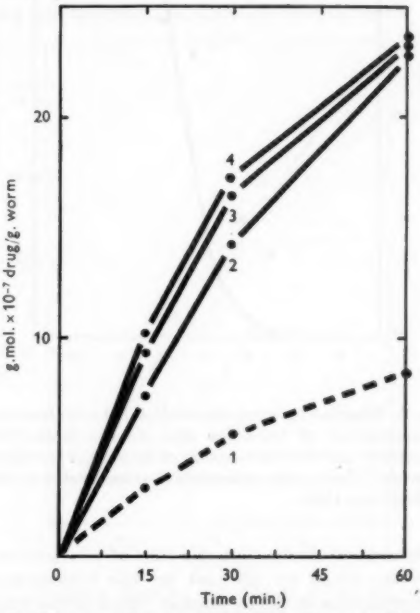


Fig. 3. Diagram showing the rates of penetration of some phenolic substances into *Ascaris* from 0·00167 M solutions. (1) 4-*n*-Butyl resorcinol, (2) thymol, (3) α-naphthol, (4) carvacrol.

Table 2. *The effect of the application of ligatures on nicotine uptake by* Ascaris

μg. nicotine/g. wet weight recovered from ten worms, average weight 4 g. at pH 6·3

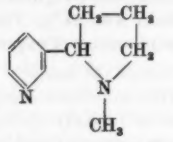
Ligatured	Not ligatured
3·4	3·6

(c) *The effect of hydrogen ion concentration and the concentration of the drug in the medium on the rate of penetration*

Nicotine (4) has two basic nitrogen groups: one in the pyrrolidine ring, pK 6·1 (Kolthoff), at 15°, and one in the pyridyl ring, pK 10·9 (Kolthoff), at 15°.

The N-methylpyrrolidine N is weakly basic and the pyridyl N is strongly basic.

(4)



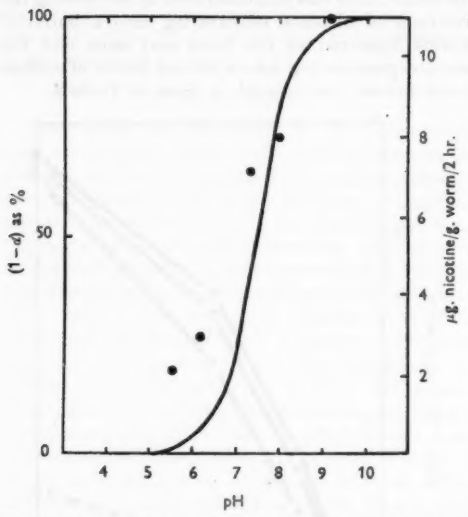


Fig. 4. Diagram showing the relation between the rate of penetration of *l*-nicotine into *Ascaris* from 0·03% solution and the dissociation of its methyl pyrrolidine group. Each point represents the mean value of thirty determinations.

It seemed likely that the rate of penetration of nicotine could be affected by the hydrogen ion concentration in the medium in virtue of the weakly basic methyl pyrrolidine group, very much as the permeability of cells to other weak electrolytes changes with the hydrogen ion concentration of the medium (see Jacobs, 1940). Fig. 4 shows the results of an experiment designed to test the extent of any such effect. Each point is the mean value of determinations made on thirty worms chosen to be approximately the same size. Table 3 gives the average weight of the individuals comprising the batches used at each hydrogen ion concentration. The measurements of hydrogen ion concentrations were made with a glass electrode at 37° C.

The smooth curve in the figure is the calculated value for $(1-\alpha)$, where α is the extent of dissociation of the methyl pyrrolidine group expressed as a percentage. The full points represent the rate of penetration of the drug. The scales are adjusted so that the point of maximal rate of penetration, 10·9 μg./g. at pH 9·1, corresponds to the full extent of association of the pyrrolidine group. It is virtually 100% at that hydrogen ion concentration. This facilitates the assessment of the effect of hydrogen ion concentration on the rate of penetration.

The values for $(1-\alpha)$ were calculated, assuming Kolthoff's (1925) value for the pK of the methyl-pyrrolidine group and Clark's (1928) value for the dissociation constant of water at 37°. It was assumed that the presence of the other electrolytes in the medium (all strong electrolytes) had no appreciable effect on the dissociation of *l*-nicotine. It was also assumed, perhaps with less justification, that the temperature coefficient of the dissociation constant of the methyl pyrrolidine group of nicotine is negligible. Tests with indicators on a 3% solution of nicotine at pH levels between 6·5 and 8·4 support this view.

Table 3. *Average weight of worms used at each hydrogen ion concentration*

pH at 37°	Buffer used	Average weight of worms used g./worm
5·5	Acetate	4·1
6·2	Phosphate (Sørensen)	4·2
7·4	Phosphate (Sørensen)	4·2
8·1	Glycine	4·2
9·1	Glycine	4·6

It appears from Fig. 4 that there is very nearly a direct proportionality between the extent of dissociation of the methyl pyrrolidine group and the rate of nicotine penetration into *Ascaris*. A similar effect has been observed by Ellisor & Richardson (1938), who studied the penetration of this drug into the goldfish. As may be inferred from Fig. 4, if the rate of penetration is plotted against $(1-\alpha)$, the points lie closely about a straight line cutting the ordinate above the origin at *c.* 2·0 μg./g. wet weight worm/2 hr., which suggests that the completely ionized base is able to penetrate at that rate.

These results suggest that the effect of the hydrogen ion concentration in the medium on the rate of penetration of the drug is a simple one concerned with the extent of dissociation of the methyl pyrrolidine radical. However, it is possible that other phenomena may contribute to the total effect. They include:

(1) The state of dissociation of the pyridyl N.

(2) The condition of the cuticular membrane, which may change with the hydrogen ion concentration.

(3) The effect of the buffering and nicotine fixing actions of the worm tissues.

Fig. 5 shows the penetration/g. wet weight at two pH levels and three different concentrations of nicotine in the medium. Fig. 6 represents the nicotine uptake over a period of 8 hr. from a solution containing 0·10 mg. nicotine/ml. at pH 8·5.

(d) The effect of substances added to the medium on the rate of penetration.

The effect of components of the environment on the rate of penetration of *l*-nicotine is clearly important in determining its efficiency as a drug. A number of substances were added to the medium, and in most cases produced no significant change in the rate of penetration. The only exceptions were certain surface active substances, which were able to produce an acceleration of the rate of penetration of the drug. Trim (1944) and Alexander & Trim (1946) showed that these also accelerate the penetration of hexyl resorcinol. The inactive substances are listed in Table 4.

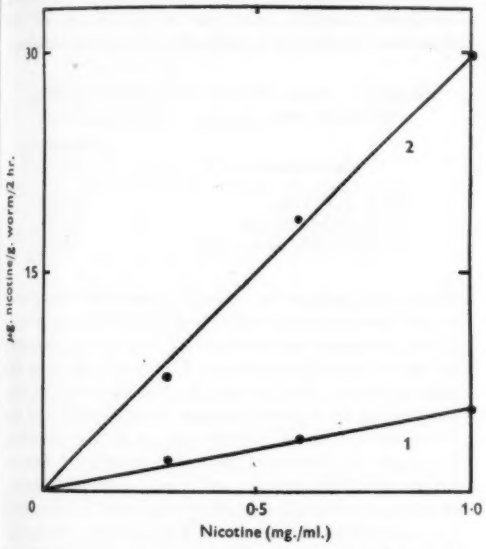


Fig. 5. Diagram showing the relation between the rate of penetration of *l*-nicotine into *Ascaris* and the concentration of drug in the medium (1) at pH 6·8, (2) at pH 8·5.

Although some surface active substances, such as sodium tauroglycocholate, have no effect on the rate of penetration of nicotine into *Ascaris*, certain other detergent substances such as C.T.A.B. (cetyl trimethyl ammonium bromide) greatly increased its action. Fig. 7 shows the results obtained by adding increasing amounts of C.T.A.B. to a 0·03 % solution of *l*-nicotine at pH 6·8; each point represents the mean value for five worms. This phenomenon has certain features in common with that obtained with mixtures of C.T.A.B. and hexyl resorcinol (Alexander & Trim, 1946). By analogy with the latter effect the following explanation may be suggested. In the early stages of increasing the soap concentration the decreasing oil/water interfacial tension of the solution leads to an increase of the concentration of nicotine in the interface between the worm and the medium and consequently an acceleration of nicotine penetration. This explanation requires the attainment of a maximal rate of penetration when the oil/water interfacial tension of the medium becomes minimal. Fig. 7 shows that there is a close correlation between the relative lowering of the interfacial tension and acceleration of nicotine penetration.

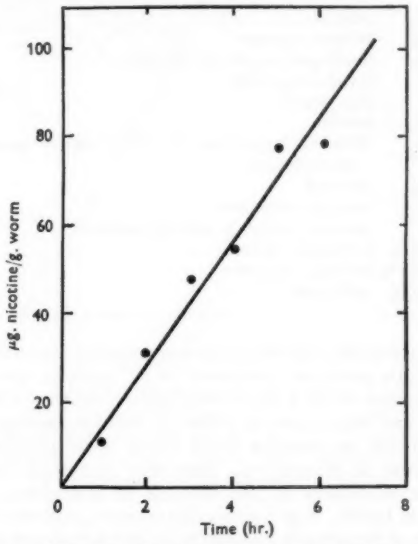


Fig. 6. Diagram showing the rate of penetration of nicotine into *Ascaris* at pH 8·5 from a 0·1 % solution. Each point represents the mean value of eight determinations.

C.T.A.B. is a cationic detergent. An equally great acceleration of the rate of penetration of nicotine into *Ascaris* was found to be produced by the anionic detergent sodium cetyl sulphate. A smaller effect was produced by a suspension of commercial egg lecithin. This may have been due to lecithin itself, which is highly surface active, or to a surface active impurity in the preparation. The quantitative relationships are shown in Table 5.

The interpretation of these results appears to be similar to that which accounts for the accelerating effects of the same substances on the penetration of hexyl resorcinol into *Ascaris* (Alexander & Trim, 1946). However, the following evidence suggests that other possibilities remain to be considered.

It can be shown by the method described below that, under the conditions of these experiments, some detergents, namely those which are able to increase the penetration of nicotine, alter the cuticle itself and increase its permeability to water-soluble substances. Hotchkiss (1944) refers to a similar effect of detergents on the permeability of bacteria, which leads to a rapid escape of organic and inorganic phosphate and nitrogenous material from the cells. Detergents also cause a loss of inorganic phosphate from *Ascaris*. Batches of four worms were ligatured at both ends and incubated at 37° with 50 ml. of a solution of C.T.A.B. in 0·9 % saline. Samples of the medium were taken at intervals and the detergent removed by shaking three times with an equal volume of chloroform. Inorganic phosphate was then estimated by the method of Berenblum & Chain (1938). Fig. 8 shows the relation between the rate of phosphate leakage from the worm and the concentration of C.T.A.B. It appears that the effect begins to occur at concentrations of C.T.A.B. between 0·003 and 0·01 %, i.e. at the concentration at which the formation of micelles of the detergent in the bulk of the medium begins (see Fig. 7 and Alexander & Trim, 1946). Removal of the medium containing detergent at this stage does not stop the loss of phosphate.

Table 4. *Substances added to the medium which do not affect the rate of penetration of nicotine at* pH 6·8

Reconstituted bovine serum	
1·0%	gelatin
1·0%	Witte's peptone
0·5%	fibrinogen (acid hydrolysate)
1·0%	starch suspension
1·0%	gum arabic
1·0%	glucose
1·0%	olive oil suspension in 1·0% sodium tauroglycocholate
0·1%	glycerol
0·5%	tauroglycocholate
0·25%	tauroglycocholate micelles, present
0·125%	tauroglycocholate
0·0625%	tauroglycocholate
0·001%	digitonin

It might be argued that this effect is not due to a change in the cuticle, but results from internal changes produced in the worms leading to a release of inorganic phosphate to which the cuticle may be naturally permeable. This is not so. A further simple experiment confirmed the fact that C.T.A.B. produces a marked loss of selective permeability in the cuticle of *Ascaris*. Cuticle stripped off the living worm may be made into a cylindrical sac by ligature at one end. When the sac is filled with a solution of a dye, such as gentian violet in 0·9 % NaCl, and suspended in a solution of 0·9 % NaCl, the dye will not pass out of the sac. If the outer solution is replaced by a 0·1 % solution of C.T.A.B. in 0·9 % NaCl the dye passes through the membrane.

Concentrations of other detergents above and below the critical level for micelle formation were tested. Cetyl sulphate produced a similar phosphate leakage to that caused by C.T.A.B.; but Na cholate, Na deoxycholate and saponin were without effect, i.e. in other words, the two detergents found to produce an accelerating effect on the rate of penetration of nicotine into the worm also cause phosphate leakage out of it. The important difference in the effects is that, whereas the increase of the rate of nicotine penetration occurs over the range of falling interfacial tension, reaching a maximum at the minimum interfacial tension, the phosphate leakage effect is initiated as the minimal interfacial tension is reached and increases greatly with further increases of the detergent concentration, i.e. as the concentration of micelles increases. This suggests that the latter effect is due in part to the removal or disorientation of a lipoid barrier in the cuticle of the worm by the lipoid-solvent action of the micelles. It cannot be assumed that the detergent has no action on the cuticle at lower concentrations, because it is rapidly adsorbed on to the cuticle at all concentrations (Alexander & Trim, 1946). Although the evidence is more in favour of the view that C.T.A.B. accelerates by the same mechanism the rate of penetration of both nicotine and hexyl resorcinol (Alexander & Trim, 1946), the accuracy of this view can only be established by further investigation. From the practical point of view it is important to recognize that there are numerous possibilities of interaction between drug and detergent, and detergent and organism, all of which may be of great importance in the application of anthelmintics.

Table 5. *Acceleration of nicotine penetration produced by some surface active substances*

Accelerator	Acceleration (%)
0·1% C.T.A.B.	1500
0·1% cetyl sulphate	1500
0·5% lecithin suspension	800

The effect of C.T.A.B. and cetyl sulphate on the rate of penetration of nicotine into *Ascaris* differs from their effect on the penetration of hexyl resorcinol (Alexander & Trim, 1946) in that no inhibition of the penetration of nicotine is produced when micelles of the detergents are present in the solution. This is probably due to the inability of nicotine to become associated with micelles. Association of substances with micelles may be regarded as the

solution of the substance in their interior which behaves like very small droplets of liquid hydrocarbon. Since the lipoid solubility of nicotine is very low it is unlikely that it has any great affinity for soap micelles.

(3) *Chloroform*

Chloroform penetrates into *Ascaris* more rapidly than any other substance studied except heptyl resorcinol. The rates of penetration from equimolar solutions are compared in Table 6.

Table 6. *Relative rates of penetration of drugs into* Ascaris *from equimolar solutions, taking the rate for nicotine as unity*

Nicotine completely dissociated	1
Nicotine at pH 9	5
Hexyl resorcinol	1240
Chloroform	1660

Penetration occurs through the cuticle and the rate is approximately proportional to the concentration of chloroform in the medium over the range 0·01–0·1 %. An equilibrium state is reached in which the concentration of chloroform in the worm is about 10 % higher than that in the medium. The drug is rapidly dispersed through the tissues. After 15 min. immersion in 0·1 % chloroform at 37° extracts of all tissues contained chloroform. The passage of chloroform out of the worm, through the cuticle, was also demonstrated by immersing worms in saline after a short immersion in chloroform solution.

As in the case of nicotine and hexyl resorcinol (Trim, 1944) a number of substances were tested for their effect on the rate of chloroform penetration into *Ascaris*. They included proteins, carbohydrates, and fatty substances and their products of digestion, bile salts and mucin. The effect of changing the hydrogen ion concentration in the medium over the range from pH 4 to pH 8 was also investigated. It was not possible to show any significant effect of these factors on the rate of penetration of chloroform.

IV. ABSOLUTE PERMEABILITIES

The area of worm surface per g. wet weight of worm used in the above experiments was 7·5–10 cm.², so that the penetration values given may be converted to approximate figures for the absolute permeability of the cuticle of *Ascaris* to the various substances used (under the conditions of these experiments). Permeability is defined as the amount of substance in g.-mol. passing through 1 sq.μ. of the membrane per sec. per g.-mol. concentration difference across the membrane. Permeabilities are recorded in Table 7.

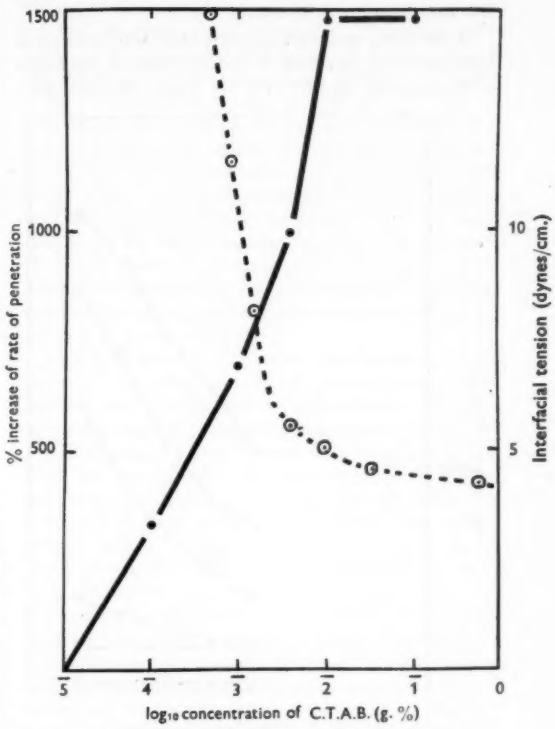


Fig. 7. Diagram showing the effect of C.T.A.B. on (*a*, dots) the rate of penetration of *l*-nicotine into *Ascaris* from a 0·03 % solution at pH 6·8 (each point represents the mean value of eight determinations); (*b*, circles) the oil/water interfacial tension of the solution (see Alexander & Trim, 1946).

V. SOME GENERAL OBSERVATIONS ON THE CUTICLE OF *ASCARIS*

Lipoid material in some form seems to play an adjusting role in the cuticle of *Ascaris*. This membrane is a predominantly protein structure (N, 16·4 %) and no lipoid has been demonstrated in it by histological methods. Extraction of the cuticle with fat solvents shows that it does contain traces of free and bound lipoid. There are three ways in which this fatty material could be distributed in the cuticle:

(1) It may be dispersed throughout the cuticle so as to be undetectable histologically.

(2) It may be present in a combined form protected from the action of fat reagents.

(3) It may be present as a very thin film on the worm's surface.

In the discussion section of this paper reasons will be given for believing that the third possibility is likely.

The homogeneity of the cuticle

It has been assumed that the cuticle of *Ascaris* is homogeneous, i.e. that it is composed of the same morphological layers over its whole surface and, consequently, that its permeability is the same at all points on the surface. However, the methods used for the measurement of permeability do not permit the detection of the existence of areas of different permeability. If areas of relatively high permeability existed close to vital organs of the parasite they would play an important part in deciding the efficiency of an anthelmintic. Some qualitative tests with triphenylmethane dyes showed that, as far as these substances are concerned, there are such areas of higher permeability located in small areas round the head, anus and (in the female) the genital pore. Random areas of relatively high permeability to these substances were also noticed. They appear to have been due to mechanical damage of the cuticle. Similar areas were produced artificially by rubbing selected areas with an ether swab. It is probable that these areas of greater permeability play the same part in the penetration of all the substances which have been studied in this work. However, their permeability appears to be only quantitatively different from that of the rest of the cuticle, because any change in the medium or the structure of the drug which brings about a changed rate of penetration also causes a corresponding change in the toxic effect.

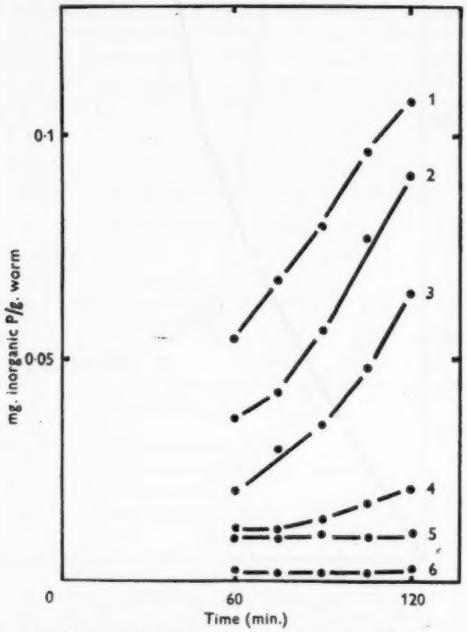


Fig. 8. Diagram showing the rate of leakage of inorganic phosphate from *Ascaris* immersed in C.T.A.B. solutions: (1) 0·1%, (2) 0·1%, (3) 0·1%, (4) 0·01%, (5) 0·003%, (6) 0·001%.

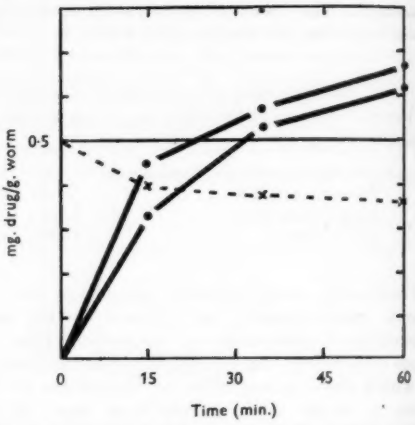


Fig. 9. Diagram showing the rate of penetration of chloroform into *Ascaris* from a 0·05% solution.

Table 7. *Permeabilities of* Ascaris *cuticle*

Substance	Mol. wt.	B, olive oil/water partition coefficient	Permeability P from experimental results ($\times 10^{-16}$)	$PM^{\frac{1}{2}}/B = C$ ($\times 10^{-16}$)	$0{\cdot}13\ B/M^{\frac{1}{2}}$ = calculated permeability ($\times 10^{-16}$)
Resorcinol	110	2·6	0·47	2·0	0·033
Propyl resorcinol	152	70	0·66	0·13	0·72
Butyl resorcinol	166	210	2·0	0·13	2·1
Hexyl resorcinol	194	1800	9·8	0·07	17·0
Heptyl resorcinol	208	5700	24·7	0·07	52·0
Thymol	150	(700)	5·0	0·13	7·4
Carvacrol	150	700	6·6	0·13	7·4
α-Naphthol	144	700	6·6	0·13	7·6
Chloroform	119	(380)	13·2	0·4	4·5
l-Nicotine^{++}	158	8	0·008	0·013	0·08
l-Nicotine^{+}	159	42	0·04	0·013	0·45

VI. DISCUSSION

The experimental results suggest that the penetration of many substances into *Ascaris* depends upon their being partitioned between the medium and a thin, outer, lipoid layer of the cuticle. The following argument will show that this generalization may be used to calculate the permeability of *Ascaris* cuticle to a large number of substances on the basis of the quantitative data given in this paper, because the toxicity of many substances to *Ascaris* is dependent mainly upon their rate of penetration through the cuticle; such calculations help us to predict their anthelmintic action. The experimental results also provide a rational basis for choosing optimum conditions for the application of anthelmintics to *Ascaris* and for devising tests of the action of drugs against other nematodes.

In order to calculate the permeability of a membrane to a substance, it is necessary to establish the precise way in which permeability is related to the physical constants of the substance. Then by resubstitution of experimental values in the equation expressing this relationship a constant for the membrane may be obtained. The permeability of that membrane to many substances may then be calculated from their physical constants and the membrane constant. If the physical constants of substances have not been measured experimentally, they may often be calculated from a knowledge of the free energies of their component radicals (Parks & Huffman, 1932).

Thus we must first investigate whether there is a simple relationship between the permeability of *Ascaris* and the physical properties of the penetrating substances.

The results given above indicate that it is likely that the three following partitioning processes may be concerned in the penetration of organic substances into *Ascaris* through its cuticle: (1) Traube capillary effects; (2) interfacial diffusion; (3) lipoid/water partition.

Assuming each of the above three possibilities in turn the values of relative permeabilities were calculated and compared with the absolute values worked out from the experimental data. It was found that the experimental and calculated values showed the closest correlation when penetration was assumed to be controlled by partition of the penetrating substance between the aqueous medium and a thin homogeneous layer of lipoid on the surface of the worm (case 3). In this case (Danielli 1941)

$$PM^{\frac{1}{2}}/B = C, \text{ a constant.} \qquad (1)$$

That is, the permeability is a function of M, the molecular weight of the penetrating substance, and B, the partition coefficient of that substance between olive oil and water. Collander (1937) has shown that the ether/water and olive oil/water partition coefficients are a sound provisional measure of the relative lipoid solubility. This has been assumed to be true for the lipoid of *Ascaris* cuticle. The figures in Table 7 show that $PM^{\frac{1}{2}}/B$ is constant within permissible limits, except in the case of resorcinol and nicotine. In Table 7 the values of B in parentheses were obtained from tables of experimental results, and the other values were calculated from them by approximating for the changes in partition coefficients produced by structural changes. By assuming the value of C for *Ascaris* (0·13, see Table 7) obtained from the experimental results the values for its permeability to various substances may be calculated by substituting for M and B in equation (1).

It is now possible to explain the striking parallel between the relative rates of penetration of substances into *Ascaris*, and the toxic effect of the same type of compounds on other organisms. From the previous argument it is clear that this parallelism does not imply that the mechanism of the interaction of the drug and the organism is necessarily the same in each of the similar cases. The experimental results for the members of the homologous series of resorcinols (Fig. 1) provide an example of the well-known homologous series effect. This had already been demonstrated in toxicity experiments on *Ascaris* made by Lamson, Brown & Ward (1935). The homologous series effect has been observed many times in the measurement of a number of different biological responses in a variety of organisms, e.g. in the toxicity of organic substances to mammals and bacteria (see Ferguson, 1939), the acceleration of the haemolytic action of lytic substances by adjuvants (Ponder & Hyman, 1939) and the reaction between an enzyme and a homologous series of substrates (Pigman & Richtmyer, 1942). Ferguson showed that, when these biological responses were measured, they bore an exponential relationship to the number of carbon atoms in the molecule as do partition properties of the homologous series concerned, so that the biological effect must be dependent upon partition properties of the series. Partition properties depend upon the free energies of the substances, and free energy is a function of structure. In the case of the experimental results under consideration in this paper the penetration of the resorcinol series into *Ascaris* must be determined by one or a number of possible partition effects. The above calculations leading to equation (1) show that it is likely that only one partition effect, lipoid/water partition, is of any great importance. In the same way the parallel homologous series effect observed by Leonard (1924) in the toxicity of the resorcinols to certain bacteria must be limited by one or more partition phenomena, and not necessarily by those which account for the effect observed in *Ascaris*.

This treatment of the permeability of *Ascaris* will

undoubtedly err in the direction of oversimplification. However, while its application will have limitations, it may be applied with most certainty to those relatively fat-soluble molecules which are generally used against roundworms. A study of the exceptions to this generalization will show the specific details in which the cuticle of *Ascaris* differs from the simple model and can open the way for the development of more efficient anthelmintic treatments.

VII. SUMMARY

1. The rate of penetration of some representative drugs into *Ascaris lumbricoides* var. *suis* has been measured.

2. The series of 4-*n*-alkyl resorcinols shows a typical homologous series effect.

3. *l*-Nicotine penetrates relatively slowly.

4. Its rate of penetration is greatly influenced by the extent of dissociation of its methyl pyrrolidine basic group.

5. The rate of penetration of nicotine is greatly accelerated in the presence of some surface active substances.

6. Chloroform is the most rapidly penetrating substance studied, and its penetration is not significantly influenced by the presence of proteins, carbohydrates and fats and their products of digestion.

7. An analysis of the experimental results shows that the outermost layer of the cuticle of *Ascaris* is probably the main barrier to penetration. It behaves as if it were a thin, homogeneous layer of lipoid.

8. The significance of these results in the study of anthelmintics is assessed.

The author wishes to thank Prof. A. C. Chibnall and Prof. D. Keilin for their interest in this work, which was a part of a programme of work on the mode of action of anthelmintics carried out for the Agricultural Research Council. He is also indebted to Dr G. Lapage, Dr E. Friedmann and Dr J. F. Danielli for their interest and help.

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ON THE GREGARINE *LANKESTERIA CULICIS* (ROSS), 1898, FROM THE MOSQUITO *AËDES* (*FINLAYA*) *GENICULATUS* (OLIVIER)

BY P. N. GANAPATI AND P. TATE

From the Molteno Institute, University of Cambridge

(With 5 Figures in the Text)

I. INTRODUCTION

Workers interested in mosquitoes and mosquito-borne diseases have, from time to time, recorded the existence of a gregarine in the gut of the 'brindled mosquito' from various parts of the world. As early as 1895 Ross (1906) observed the parasite in the 'Stegomyiae' collected from various parts of India, and he named it *Gregarina culicis*. Marchoux, Salimbeni & Simond (1903), working in South America, observed in broad outline the various stages of the gregarine leading to spore formation, when the host *Aëdes aegypti* developed from the larva to the adult. Wenyon (1911) made a detailed study of the form, from the same host collected from wells in Baghdad, and named it *Lankesteria culicis* (Ross), on account of the many points of resemblance between his form and *L. ascidiae*, described by Siedlecki (1899) some years earlier from the ascidian *Ciona intestinalis*. Stevenson & Wenyon (1915), Bacot (1916), and Macfie (1917) recorded the gregarine from West Africa, while its range of distribution was extended to North China with the discovery of the parasite by Lan-Chou (1930) in the gut of *Aëdes koreicus*. Ray (1933) studied in some detail the development of the parasite which he discovered in the gut of *A.* (*Stegomyia*) *albopictus*, collected at Calcutta, India; and while confirming, in the main, the observations of Wenyon, he found some new features in the light of which he concluded that its present position among the haplocyte gregarines is anomalous, and suggested that its proper place would be among the Cephalina or septate gregarines, where a separate genus may have to be created for it.

A. geniculatus was a constant denizen of a hole in a horse-chestnut tree in the garden of the Molteno Institute, associated with several other dipterous larvae (Keilin, 1927, 1932). In the summer of 1931 many of the larvae and pupae were observed in a dying condition. When brought into the laboratory they were lying at the bottom of the vessel making spasmodic strokes with the abdomen. On examination these larvae and pupae were found to be heavily infected with the various stages of a gregarine which proved to be the same parasite, *Lankesteria culicis*, recorded by other workers from other species of *Aëdes*. Although the host *A. geniculatus* is a very common arboreal mosquito in the southern counties of England and throughout Europe, there appears to have been no previous record of the parasite from this host or from Europe. It is also interesting as it adds another to the list of nine protozoan parasites recorded by Keilin (1927) for the same tree hole from other dipterous larvae. Apart from the extension of its range of distribution to this country there are several interesting features connected with its morphology and systematic position which we propose to review in the light of our observations and those of previous workers.

II. MATERIAL AND METHODS

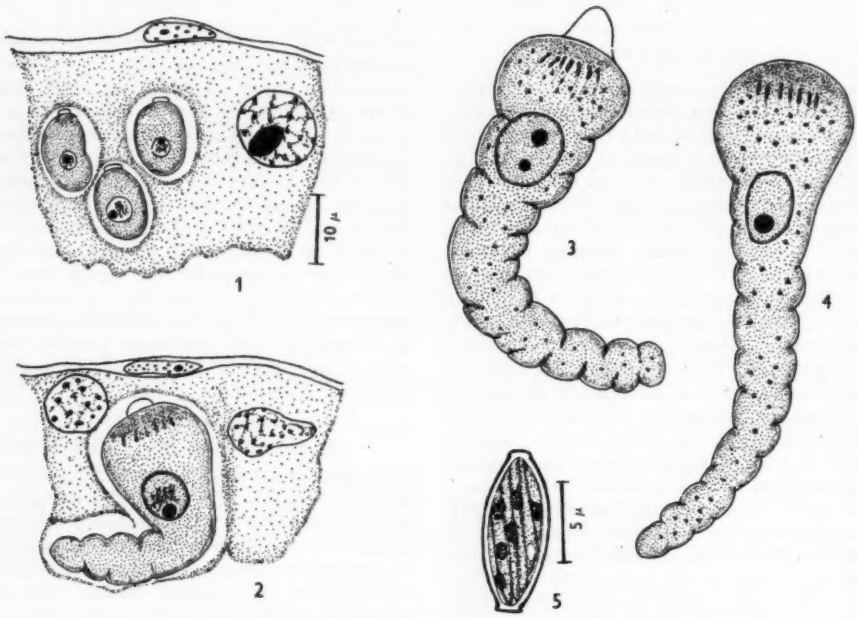
The larvae, pupae and adults were collected on three successive days in July 1931. The living parasites were observed by teasing out the gut contents in normal saline or Ringer's fluid. Smears were fixed in Schaudinn's solution and stained with Delafield's haematoxylin or Mann's stain. Material for sectioning was fixed in Bouin's fluid. Serial sections of entire larvae, pupae and adults were made and stained with Heidenhain's iron-alum haematoxylin. Light green was used as counter stain whenever necessary.

III. MORPHOLOGY AND LIFE CYCLE OF THE GREGARINE

The gregarine infects the gut of the host, and has not been seen in any other organ. The youngest forms, measuring $10 \times 7\ \mu$, were intra-epithelial in the oesophagus and anterior mid-gut of the larvae (Fig. 1). The body is ovoidal and at the anterior end, directed away from the gut lumen, is the epimerite in the form of a button, which does not stain in sections or smears. The part of the main body segment immediately behind the epimerite stains more deeply than the rest of the body cytoplasm, and contains a few deeply staining granules which are basophilic

in nature. The nucleus is spherical with a conspicuous eccentric karyosome. Both Wenyon (1911) and Ray (1933) have drawn attention to the deeply staining anterior zone of the body segment, and the latter considers this to be a rudimentary protomerite which in septate (Cephaline) gregarines is separated by a septum from the rest of the body or deutomerite. Marchoux *et al.* (1903) did not observe either epimerite or protomerite in the form they studied. In sections the parasites are seen to lie in vacuoles in the epithelial cells with the anterior ends always directed away from the gut lumen. Growth, mainly in length, takes place in the region immediately behind the nucleus and this part of the body presents a ringed appearance, a very characteristic feature of the trophozoites which persists even in the free forms after liberation from the gut epithelium (Fig. 2). The anterior end is broad and truncated, while posteriorly the body gradually tapers to a blunt extremity. When the parasites attain a length of about 50 μ they are liberated into the gut lumen. The free trophozoites attain a length of 100–150 μ and are not much differentiated from the fully grown intra-epithelial stages. The protomerite contains a larger number of basophilic granules and, on careful examination, radiating fibres converging on to the epimerite may be made out (Fig. 3). The epimerite persists in the free forms and functions as a sort of sucker for attachment to the epithelium.

There is some diversity of opinion regarding the nature and function of the epimerite in *Lankesteria culicis*. Wenyon (1911) describes it as 'a vesicle... possibly a suction organ connected with the endoplasm through a pore in the cuticle'. According to this author the epimerite serves as an organ of fixation to the remnants of the epithelium when the parasite outgrows and ruptures the host cell. The epimerite is also described as holding together the two sporonts at the time of association prior to cyst formation. Our observations have in the main substantiated Wenyon's description. We, however, believe that in view of the motility of the trophozoites, attachment need not necessarily be to the remnants of the affected cell but may be to any part of the epithelium. This may be the explanation why



Figs. 1–5 of *Lankesteria culicis* (Ross) from *Aëdes geniculatus* (Olivier).

Fig. 1. Intracellular stages in epithelial cells of gut.
Fig. 2. Fully grown intracellular parasite.
Fig. 3. Free trophozoite from the lumen of the gut.
Fig. 4. Sporont.
Fig. 5. Sporocyst.
Figs. 1, 2 and 5 are from sections and Figs. 3 and 4 from smear preparations. Figs. 1–4 are drawn to the same scale.

the epithelium with numerous attached parasites figured by Lan-Chou (1930) appears normal and uniformly thick. Ray (1933) found that the degree of development of the epimerite depended on the region of the gut inhabited by the parasite. According to this author the parasite undergoes two types of early development. Those which succeed in piercing the anterior part of the mid-gut and oesophagus where the chitinous lining is thin lead an intra-cellular existence with feebly developed epimerites, whereas those that do not succeed in piercing the gut anteriorly attach themselves to the posterior part of the mid-gut where the chitinous lining is thick, and lead an extra-cellular life with well-developed epimerites. The epimerite is shed at the time of liberation from the epithelial attachment. According to this author association is lateral and not end to end as observed by Wenyon.

We have not observed associating forms. The cysts and stages in spore formation were found in the malpighian tubules of the pupae. Cysts are spherical and are 80–100 μ in diameter. The sporocysts are navicular with the two poles flattened and measure $12 \times 4\,\mu$ in the fresh condition. The two poles stain deeply with haematoxylin (Fig. 5). The sporozoites are arranged along the longer axis of the sporocyst. A cystal residuum is observed in fresh spores. When the imagines emerge from the pupae the malpighian tubules are packed with the ripe spores which escape through the alimentary canal.

All authors are agreed that infection of the larvae takes place through the ingestion of sporocysts. In a tree-hole breeding form, like the present host, the chances of infection of fresh broods of larvae in a confined place are indeed great. Several larvae and pupae of two other genera of mosquitoes found in the same tree hole, *Anopheles plumbeus* and *Orthopodomyia pulchripalpis*, were examined for the possible presence of the gregarine, but always with negative results. Lan-Chou and Ray failed to infect species of *Culex* with sporocysts of *Lankesteria culicis*. It seems therefore that *L. culicis* is specific to species of mosquitoes belonging to the genus *Aëdes*.

IV. SYSTEMATIC POSITION AND AFFINITIES

It has been the practice to refer the gregarine from species of *Aëdes* to the genus *Lankesteria* ever since Wenyon (1911) pointed out the many striking similarities between the form observed by him in *Aëdes aegypti* and *Lankesteria ascidiae* described by Siedlecki (1899) from the ascidian *Ciona intestinalis*. Both forms are intra-cellular in their early stages, possess the same kind of epimerite, show a deeply staining 'protomerite' just behind the epimerite and associate by their anterior ends by apposition of sucker-like epimerites. Bhatia (1930), reviewing the genera and proposing a new system of classification of the haplocyte (Acephaline) gregarines, included *Lankesteria* in the family Diplocystidae Bhatia. Ray (1933), from a study of the parasite as it lives in *Aëdes albopictus*, came to the conclusion that the position of *Lankesteria culicis* among the haplocyte gregarines is no longer justified. According to this author the presence of a well-developed epimerite, a rudimentary protomerite, and the capacity for a completely extra-cellular development are all points strongly in favour of its inclusion among the septate (Cephaline) gregarines. He suggests the creation of a new genus, if necessary, to accommodate it there.

While, in our opinion, it shows both acephaline and cephaline features, the nearest ally of *Lankesteria* is the genus *Lecudina*, which is included in the family Lecudinidae Kamm emend. Reichenow, 1929. Lecudinidae occupies an intermediate position between the true acephaline gregarines and the cephaline (septate) forms (Ganapati, 1946). *Lecudina*, the type genus of the family, is a true dicystid, like *Lankesteria*, in which the body shows only two segments, an epimerite and a main body segment. In all the species carefully studied a rudimentary protomerite is demarcated from the rest of the body by its different staining reaction in permanent preparations and its hyaline nature in the fresh condition, though an actual septum separating these two regions is not present. The epimerite is simple and functions as a sucker. The earlier stages are intra-epithelial. Of the seven genera included in the Lecudinidae the sporocyst structure is known only in *Lecudina*. The inclusion of others is based on similarities in other morphological features, habitat and host. From the above brief summary of the salient features of the family Lecudinidae it is clear that *Lankesteria* is as much a dicystid as *Lecudina* and that it shares with the latter most of the morphological and developmental features. While the sporocysts in both the genera are simple, those of *Lecudina* show a characteristic thickening at one pole. According to our view, while the sporocyst structure may have fundamental importance in differentiating genera, it should not override other morphological similarities, and preclude the possibility of assigning *Lankesteria* to the family Lecudinidae, as it is at present constituted.

SUMMARY

1. The morphology and life history of a gregarine *Lankesteria culicis* (Ross), as it lives in the gut of *Aëdes* (*Finlaya*) *geniculatus* (Olivier), are described. The earlier stages are intra-epithelial in the fore and anterior mid-gut of the larvae. After a period of growth the trophozoites are liberated into the gut lumen where they attach themselves to the epithelium by a well-developed epimerite which functions as a sucker. Cyst formation and further stages in

sporogony take place in the malpighian tubules when the larvae develop into the pupae. In the adult mosquito only ripe sporocysts are found, packed in the malpighian tubules and scattered in the hind-gut. The spores escape through the alimentary canal.

2. The systematic position of *Lankesteria* is reviewed. In view of its true dicystid nature, combining both acephaline and cephaline features, it is suggested that its proper systematic position would be in the dicystid family Lecudinidae Kamm emend. Reichenow, 1929, which includes all forms intermediate in position between the monocystids and the true tricystids.

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THE DISTRIBUTION OF CIMICIDAE (HEMIPTERA) IN THE ANGLO-EGYPTIAN SUDAN

By D. J. LEWIS, M.A., *Medical Entomologist*,
Stack Medical Research Laboratories, Khartoum

(With 4 Figures in the Text)

INTRODUCTION

Cimex hemipterus F., the principal bed-bug in the tropics, was first recorded in the Sudan as *C. rotundatus*, by Balfour (1911*a*) on pilgrims from the Yemen at Port Sudan and Suakin, and at Loka in the southern Sudan. He stated that *C. lectularius* L., the principal bed-bug in the temperate countries, was the only species at Khartoum and in the Sennar, Kassala and Kordofan areas. At that time *C. lectularius* was little known in Africa (Howard, 1912), but later Bequaert (1930) wrote that *C. hemipterus* was the common bed-bug of Equatorial Africa and *C. lectularius* the common species in South Africa.

It is now known (Mellanby, 1935) that *C. hemipterus* has spread to many tropical countries. The Sudan is a large area which lies entirely within the tropics but has, for many centuries, had contacts with the Mediterranean area through caravans and other routes of communication. The distribution of the two species of *Cimex* promised to be of some interest, and accordingly the writer has taken the opportunity of collecting specimens (usually a few at each village) while travelling in the Sudan during the last ten years. He is very much indebted to those, whose names are mentioned in the distribution lists, who have kindly sent specimens.

An early reference to bed-bugs in the Sudan was made by De Cadalvène & de Breuvery (1836), who recorded the names of these insects in the Mahass and Dongola languages of the northern Sudan. Gordon (Hill, 1885) remarked on the apparent absence of bed-bugs near Kerri, south of Juba.

DISTRIBUTION IN THE SUDAN

After a little collecting in the Nuba Hills, the Malakal area, and some districts farther south, it became evident that in these areas *C. hemipterus* was the bug of most of the local native inhabitants and *C. lectularius* the bug of people of northern origin or of local people who had much contact with them. Such local people lived in or near towns or in villages (such as some 'arabicized' Nuba villages) which had much contact with travellers. This tendency of the two bed-bugs to be associated with the two classes of people was so marked that one could usually predict which species would be found in a particular village, and in one case (Keili) the writer was able to predict the finding of *C. hemipterus* in an area 260 miles distant after reading the history of the tribe which lived there. In doubtful cases *C. lectularius* was usually found.

In areas where both species existed *C. lectularius* tended to occur along roads and navigable rivers and *C. hemipterus* in the surrounding country. The former would occur in towns or large villages, such as Delami, Heiban, Rashad and Sources Yubu, and the latter in huts on the periphery. As a result of this distribution *C. lectularius* was collected more frequently than *C. hemipterus*. The two bed-bugs were seldom found in one house.

One could not avoid the conclusion that *C. lectularius* had probably spread into the south mainly during the last century or so, through the incursions of slave traders and other travellers. It is interesting to note that Livingstone associated the spread of bed-bugs with the slave trade in Central Africa (Waller, 1874, p. 33), and that Schweinfurth (1873) refers to a connexion between the slave trade and the spread of 'vermin' when writing of the Dinka tribe in the Sudan.

An interesting instance of the spread of *C. hemipterus* was observed in one area, that of the Tagoi Hills, which is inhabited by Nubas who lived for centuries protected from the plain dwellers by defensive walls encircling the villages. After the advent of settled conditions in the present century many migrated to the plains and took *C. hemipterus* to the new villages which they founded.

Where the name of the collector is omitted in the following records the insects are specimens obtained by the writer.

Cimex hemipterus

The known distribution (Fig. 3) is as follows: Adall, Aweng, Gogrial (*Dr H. D. Fairman*); Kapoeta (*Medical Officer*); Karnak (20 km. south of Delami, *A. A. Muhammad*); Dungunab, Gebeit Gold Mines, Muhammad Qol (*M. A. Muhammad*); Port Sudan,

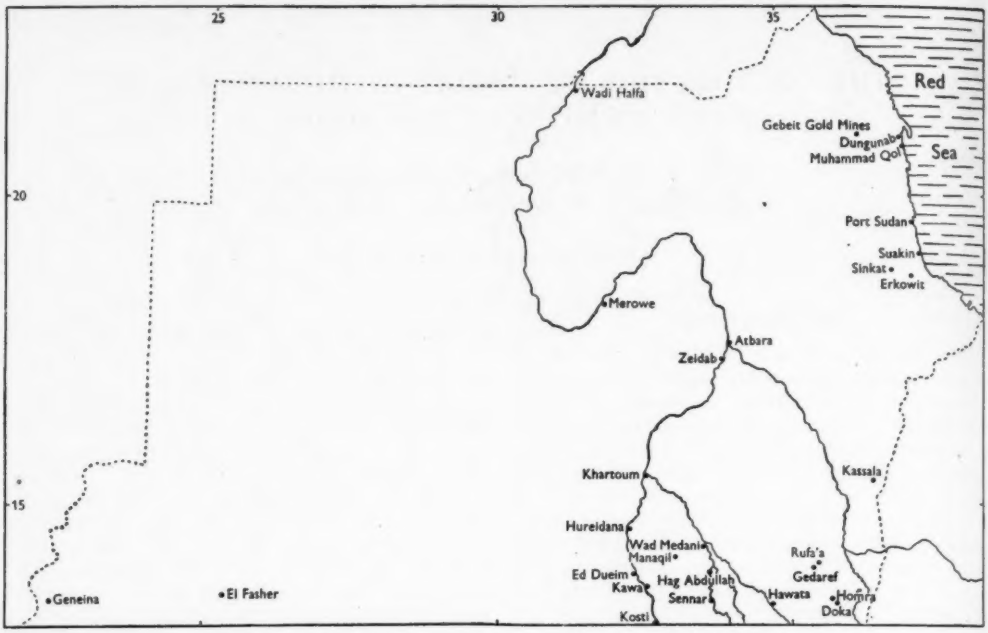

Fig. 1. The northern Sudan, map showing places mentioned in the text.

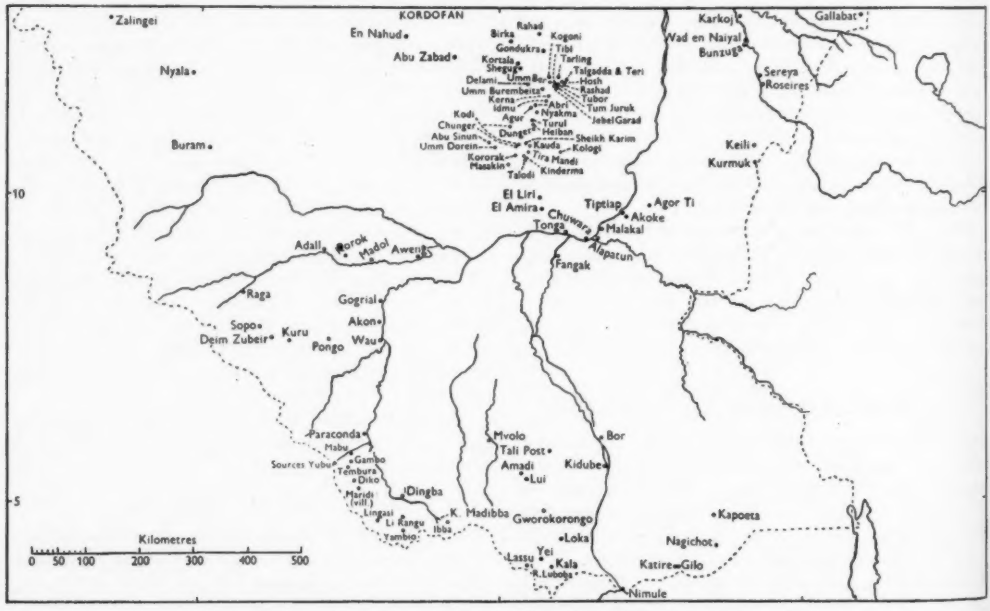

Fig. 2. The southern Sudan, map showing places mentioned in the text.

Sinkat, Suakin (*Public Health Inspector*); Agor Ti, Akoke, Amadi, Chuwara, Deim Zubeir, Diko, Dingba, Gambo, Idmu, Jebel Garad, Kala, Kerna, Khor Madibba, Kodi, El Liri, near R. Luboba, Mabu, Maridi, Masakin, Nagichot, Nyakma, Paraconda, Pongo, Sheikh Karim, 10 km. north of Sources Yubu, Talgadda, Tembura, Teri, Tibi, Tiptiap, Tubor, Turul, Umm Dorein. Archibald & Mansour (1937) mentioned *C. hemipterus* from the southern Blue Nile area. Both *C. hemipterus* and *C. lectularius* have been found at the following places: Keili (*H. M. Ali*); Port Sudan (*J. C. Edwards*); Sources Yubu (*Dr A. R. Hunt*); Abri, Chunger, Erkowit, Heiban, Hosh, Li Rangu, Loka, Malakal, Tum Juruk, Umm Ber, Yei.

Sinun, Agur, Alapatun, Amadi, El Amira, Birka, Ed Dueim, Dunger, Fangak, Gilo, Gondukra, Hag Abdullah, Hawata, Homra, Hureidana, Ibba, Idmu, Katire, Kawa, Kidube, Kinderma, Kodi, Kogoni, Kologi, Kororak, Kortala, Kosti, Lassu, Lingasi, Lui, Manaqil, Merowe, Mvolo, Nimule, Rahad, Rashad, Roseires, Rufa'a, Sereya, Shegug, Talodi, Tali Post, Tira Mandi, Tonga, Umm Burembeita, Wad Medani, Wadi Halfa, Yambio.

C. lectularius is widespread in the inhabited parts of the northern Sudan, except near the coast, and is widely distributed along lines of communication in the south. It is the common bed-bug in many places 800 miles south of the Tropic of Cancer and not uncommon 1100 miles south.

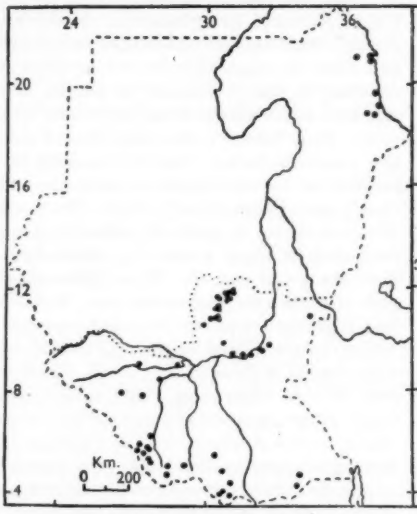

Fig. 3. The known distribution of *Cimex hemipterus*; the dotted line represents the boundary between the northern and southern Sudan as defined in the text.

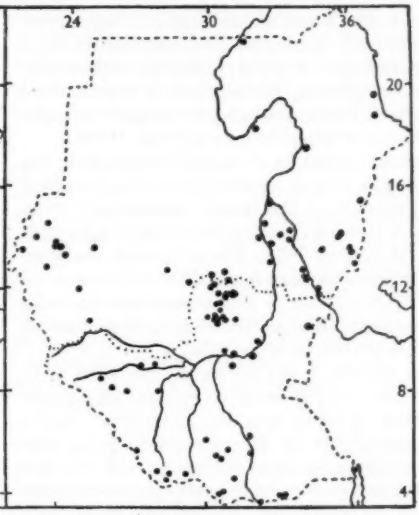

Fig. 4. The known distribution of *Cimex lectularius*.

C. hemipterus is widespread in the southern Sudan and along the coast over 500 miles further north.

Cimex lectularius

In addition to the places mentioned above at which both species were obtained, this bed-bug has been found at the following (Fig. 4): Kodok, north of Akoke (*A. K. Abdullah*); Bunzuga, Kurmuk (*Ali*); Khartoum (Balfour, 1911*a*); Gallabat, Gedaref, Kassala (Bousfield, 1908); Akon, Korok, Kuru, Madol, Raga, Sopo, Wau (*Fairman*); Abu Zabad, Buram, Doka, El Fasher, En Nahud (*N. M. Ghanim*); Geneina, Nyala, Zalingei (*Dr E. W. T. Morris*); Kebkabiya, Otash, Sireif, Jebel Asam, J. Meilo, J. Shoba (all near Geneina, *A. A. Muhammad*); Karkoj, Wad en Naiyal (*Dr W. F. Townsend-Coles*); Abu

Along the Nile between Merowe and Wadi Halfa *C. lectularius* appears to be rare or absent in most places. At Merowe, after much searching, it was found in a house in a damp heavily shaded garden. At Khandaq it is said to have been recently introduced or reintroduced by travellers. Hoskins (1835), commenting on an observation by Burkhardt (1822) on the absence of 'vermin' from leather-covered bedsteads in a northern area, attributed this to the heat of the climate.

Leptocimex boueti Brumpt.

L. boueti has been found at Zeidab (November 1935, *W. P. L. Cameron*) and at Roseires (July 1945, a few individuals on the walls of a bat-infested house). It is probably more widely distributed.

OTHER NOTES ON BED-BUGS

In one or two individuals, among thousands examined, the shape of the prothorax was intermediate between *Cimex hemipterus* and *C. lectularius*.

Bed-bugs are sometimes very numerous in certain areas. The writer has seen about a thousand on a single bedstead in the Nuba Mountains, and Maurice (1932) in Darfur saw a bedstead, left in the sun, on the shaded part of which there were so many bugs that they resembled overlapping scales, and he thought at first that the wood was covered with snake skin. In the central Sudan bed-bugs can usually be found with little difficulty except in the hot dry season. They have never been found in encampments of nomads.

Bed-bugs often travel from village to village. They have been found carried on clothing, furniture, a bandolier, an ambatch-wood shield, and in steamers, and have occasionally been found in trains. One specimen of *C. hemipterus* has been taken in an aeroplane arriving at Khartoum (Whitfield, 1939).

The common practice of placing bedsteads in the sun to kill bugs is often an effective means of control in areas where even the shade temperature often exceeds the thermal death-point of bed-bugs which is 44° C. (Mellanby, 1935). The bedsteads are often beaten with sticks so that bugs fall to the hot ground and die in a few seconds. Sometimes bedsteads are sunk in rivers to kill bugs. In towns modern residual insecticides are becoming popular.

Balfour (1908*a*, *b*) fed *Cimex* sp., recorded as *C. macrocephalus*, on jerboas and fowls in experiments on parasites of these animals. Archibald (1911), during experiments on *Herpetomonas lygaei*, kept *Cimex lectularius* confined with a lygaeid bug and observed that the former appeared to feed on the latter.

Bed-bugs in the Sudan are also mentioned by Aders (1911), Archibald (1914, 1917, 1923), Balfour (1911*b*), Balfour & Thomson (1911), Bousfield (1911*a*, *b*), Cummins (1908), King (1908), Marshall (1911) and Thomson (1911), mainly in relation to attempts to transmit various diseases.

DISCUSSION

Mellanby (1935) discussed the distribution of the two species of bed-bug in relation to their physiology. He considered that distribution depended largely on opportunities for dispersal and that *C. lectularius* was likely to spread in the tropics where it was introduced. Physiologically the two species have many resemblances; one of their differences is the tendency of *C. lectularius* to resist desiccation. Bequaert (1926) considered that some climatic factor prevented the establishment of this species in moist equatorial regions.

We may consider the distribution of bed-bugs in the Sudan in the light of (1) facilities for dispersal, (2) the probability that *C. lectularius* is better adapted than *C. hemipterus* to very dry conditions, and (3) the supposition that *C. hemipterus* is better adapted to life in very humid tropical areas.

It is known that during the last few thousand years the northern Sudan has been the scene of periodical movements of people of various origins, some from Egypt and other Mediterranean areas and some of Negroid origin. The present population is largely of mixed ancestry with evidence of Negroid blood in many areas. For a long period there was little penetration from the north into the southern Sudan, which is to a great extent separated from the north by the vast mosquito-infested swamps of the Upper Nile and its tributaries. In the nineteenth century slave raiders penetrated far into the south, and later the establishment of Government control resulted in the movement of people of northern origin to many places along rivers and roads in the south. Thus there is now a large area of the country, the 'southern Sudan', inhabited mainly by Negroid peoples but containing numerous towns and villages closely associated with the north. The boundaries of this area cannot be precisely defined but are roughly indicated in Figs. 3 and 4 as including the two southern provinces, the Nuba Hills districts and, east of Renk, the Ingessana area. The Nuba Hills and Ingessana area are included because their inhabitants are placed in the southern Sudan in the ethnological section of *The Anglo-Egyptian Sudan from Within* (Hamilton, 1935), a book which gives much information on the history and present condition of the country. In the nineteenth century the inhabitants of the Nuba and Ingessana Hills were able to hold out against hostile northern forces which overran the surrounding plains and their inhabitants form outliers, so to speak, of southern people surrounded by northerners.

Places on the Red Sea coast have for long been in contact with the outer world by sea. Suakin, which has yielded its importance to the comparatively new Port Sudan, is at least 500 years old, and probably originated from a trading settlement started over 2000 years ago. Balfour attributed the presence of *C. hemipterus* on the coast to immigration from the Yemen, but many opportunities must have occurred for the introduction of both species, from the west by land, and the north, east and south by sea.

From these brief notes on the history of the country we may consider what would be the expected distribution of the two bed-bugs if opportunity for dispersal were the only determining factor, assuming that *C. hemipterus* originated in the tropics and *C. lectularius* in the temperate zone. We should expect to find *C. hemipterus* widely distributed in the south and in many parts of the northern Sudan,

and *C. lectularius* in all inhabited parts of the northern Sudan and along lines of communication in the south. The actual distribution is largely as would be expected, and the above-mentioned association of *C. lectularius* with people of northern tribes living in the south supports the supposition that opportunity for dispersal is an important factor.

The differences between the actual and the expected distribution are the absence of *C. hemipterus* from the inland northern area and the scarcity of *C. lectularius* on the coast. The effect of differences in atmospheric humidity may be responsible; the humid coastal atmosphere may favour *C. hemipterus*, and the arid climate of the interior of the northern Sudan may have affected *C. hemipterus* more severely than *C. lectularius*. Even the latter species evidently lives under very adverse conditions in the far north.

Other factors may affect the distribution of the species. For example, the situation on the Red Sea coast may be comparable to that on Formosa where Omori (1941) considered that an injurious effect of the spermatozoa of *C. hemipterus* on females of *C. lectularius* might be responsible for the failure of the latter species to establish itself on the island. It is difficult to estimate the effects of temperature in the nothern Sudan where great heat as well as somewhat low temperatures are experienced. The dispersal of *C. lectularius* is assisted by its association with northerners who travel farther and carry more luggage than southerners.

SUMMARY

1. The distribution of *Cimex hemipterus* and *C. lectularius* in the Sudan is described. *Leptocimex boueti* has been found in two localities.

2. Reasons for the distribution of the two species of bed-bug are discussed, and it is considered likely that facilities for dispersal and atmospheric humidity may have been the most important factors.

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THE WATER-SOLUBLE VITAMINS OF PARASITIC WORMS

By M. R. A. CHANCE and P. DIRNHUBER
Pharmacology Department, Medical School, Hospitals Centre, Birmingham

While investigating the nutrition of parasitic helminthes it became possible to estimate the amounts of several members of the vitamin B complex in the parasites. Information on this point was required in order to obtain some idea of the composition of a satisfactory *in vitro* diet.

Samples of the worms were prepared for assay by washing in at least three lots of tap water; large worms were rubbed with a damp cloth to remove material adhering to the external surface. *Nippostrongylus* worms obtained in bulk were washed free of mucus. After weighing, the samples were desiccated, either by heating at 70° C. in vacuum or by freeze-drying: both procedures reduced the weight by 70–80%. The dried powders were suspended in water and assayed by a microbiological procedure. Both standard and test sample were tested on the appropriate micro-organism at such levels as to give four different points on the linear part of the curve relating response to log dose.

The micro-organisms used for the assays of the different vitamins were: for aneurin, *Lactobacillus fermenti* 36; for nicotinic acid, *Lb. arabinosus*; for pantothenic acid and riboflavin, *Lb. casei*, and for pyridoxin (Siegel, Melnick & Oser, 1939 and Braunstein, Kritzmann, Gale, Tomlinson, Kritzmann & Samarina, 1946), after acid hydrolysis with N-HCl and autoclaving at 30 lb. pressure for 15 min., *Kloeckera brevis*.

RESULTS

Table 1 shows the amounts of three vitamins found in *Fasciola hepatica*. In Table 2 are set out the vitamin contents of dried populations of the larval and adult stages of *Nippostrongylus muris*. The amounts of the three B_2 vitamins in *Ascaris lumbricoides* are shown in Table 3. All the results are summarized in Table 4, together with the aneurin and pyridoxin contents of single samples of the worms.

Table 1. *B_2 vitamins in* Fasciola hepatica (*Bovis*)

Size of specimens	No. of worms	Mean content in μg./g. dry weight: Nicotinic acid	Riboflavin	Pantothenic acid
Small	4	—	6·0	14·4
Medium	9	310	2·2	9·6
Large	2	218	8·0	6·4

Table 2. *B_2 vitamins in* Nippostrongylus muris

Stage of life cycle	No. of rats	Mean content in μg./g. dry weight: Nicotinic acid		Riboflavin		Pantothenic acid	
Young larvae	—	320	260	60	35	24	19
	—	200		10		14	
Worms, 5th day of infestation	1	190	145	80	65	—	
	1	130		26		—	
	1	220		95		—	
	1	140		69		—	
	14	110		30		16	
Worms, 15th day of infestation	11	300		260		23	
	22	78		34		15	

DISCUSSION

The results demonstrate that all the vitamins tested are present in quantities comparable with those found in the tissues of higher animals, and thus it is reasonable to assume that the vitamins studied are required by the parasite. Preliminary experiments including these vitamins in a synthetic diet gave promising results with *Ascaris*.

When the amounts of vitamins in the parasites are compared with those in single samples of liver from the host animals, the quantities in the parasites are, with the exception of pyridoxin, found to be somewhat less. The values for the liver contents agree reasonably well with the reported results of microbiological assays on dried samples of liver from animals of the same species (University of Texas). As a result it is possible to use those values for comparison with those found by us. Thus, in the first instance, it would appear that the amounts of pyridoxin in the parasite are higher than those in the host's liver or the host's tissue on which it feeds. It is possible, therefore, that both *Fasciola*, which feeds on blood (Stephenson, private communication), and *Nippostrongylus*, which feeds on the gut wall and/or blood, store pyridoxin, since both these tissues contain much less pyridoxin than liver. Microbiological synthesis of some vitamins of the B group occurs in the lower end of the small intestine (Benesch, 1944), and *Ascaris* may obtain adequate supplies direct from this source. Since it is known that pyridoxin is concerned with protein synthesis, it is possible that the relatively high content of this vitamin is associated with the high rate of egg production in these parasites. The fact that the content of pyridoxin is low in the anterior proglottids of the sheep's tapeworm may mean that the tapeworm does not store pyridoxin, as the developing proglottids can obtain their vitamin requirements, along with other nutritional components, from the intestinal lumen. In view of this possibility, it is suggested that further work along these lines might be profitable, especially work in which the chemical constituents of the developing proglottids of tapeworms are involved.

Table 3. *B_2 vitamins in* Ascaris lumbricoides (*Suis*)

Size and sex of specimens	No. of worms	Content μg./g. dry weight: Nicotinic acid	Riboflavin	Pantothenic acid
Small, ♀	1	270	10	11·2
Large, ♀	2	171	18	11·2
Medium, ♂	3	168	13	51·2

Table 4. *B vitamins in parasitic worms*

Systematic classification of parasite: Class	Species	Content μg./g. dry weight whole worm: Aneurin	Nicotinic acid	Pantothenic acid	Pyridoxin M.B.	Pyridoxin C.*	Riboflavin
Nematoda	*Nippostrongylus muris* (*Mus*)†	20·0	119 (383)	19·0 (238)	13·0	15·5 (10)	84·0 (41)
Nematoda	*Ascaris lumbricoides* ♂ and ♀ (*Sus*)†	— 10·0	220 168 (600)	11·2 51·2 (55)	20·0 —	17·5 — (13)	14·0 13·0 (50)
Trematoda	*Fasciola hepatica* (*Bos*)†	16·0	293 (430)	9·0 (91)	29·0	33·5 (14)	3·25 (52)
Cestoda	*Moneizia benedeni* (anterior proglottids) (*Os*)†	8·6	190	10·0	7·1		No assay

* Barium salt of phosphopyridoxal containing 32 % pyridoxal and 50 % barium.
† Generic name of host. (Figures for vitamin contents of livers of host animals are in parenthesis.)
M.B. = Microbiological assay.
C. = Chemical estimate.

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GLYCOLYSIS AND RELATED PHOSPHORUS METABOLISM IN PARASITIC NEMATODES

By W. P. ROGERS and MARIAN LAZARUS, *Australian Council for Scientific and Industrial Research, McMaster Laboratory, Sydney, N.S.W.*

(With 6 Figures in the Text)

INTRODUCTION

The occurrence of large amounts of glycogen in the tissues of adult nematode parasites has interested many workers who have examined the rate of utilization of carbohydrate reserves in *Ascaris lumbricoides*, *Parascaris equorum* and *Eustrongylides ignotis* (e.g. see v. Brand, 1937; Toryu, 1935). However, the breakdown of glycogen has not been examined in detail beyond the work of v. Brand (1934), who determined changes in glycogen content, oxygen uptake, carbon dioxide, lactate and valerate production in starving *Ascaris lumbricoides* kept *in vitro*. The present work attempts to follow the breakdown of glycogen as far as lactate and to examine the chief intermediary reactions. The results are presented to show: (1) the effect of exogenous carbohydrate on the respiratory activity of whole organisms; (2) the formation of ester phosphate; (3) the synthesis of glycogen from Cori ester; (4) the synthesis and breakdown of fructose phosphate; (5) acid formation and bicarbonate utilization in cell-free extracts; (6) glycogen utilization, glucose and lactate formation; (7) the occurrence of phosphate compounds in resting tissues.

BIOLOGICAL MATERIALS

The nematodes, *Ascaris lumbricoides*, *Ascaridia galli*, *Neoaplectana glaseri* and *Nematodirus* spp. were obtained and their tissues prepared as described previously (Rogers 1948; Rogers & Lazarus 1948). Brei was usually prepared by mincing frozen material in a Latapie mincer and by grinding with quartz sand with a pestle and mortar in an equal weight of water. Perienteric fluid was usually removed from *Ascaridia galli* before grinding. Cell-free extracts were prepared by centrifuging tissues which had been crushed in a glass bacterial mill.

Materials used for the determination of phosphorus compounds in resting tissue were treated with care to avoid the breakdown of unstable substances. Wherever possible, samples were kept frozen and manipulations were carried out as quickly as possible.

METHODS

The phosphate compounds soluble in trichloracetic acid

Acid-soluble phosphate was extracted with trichloracetic acid and fractionated according to the solubility of the barium salts in alcohol (see Umbreit, Burris & Stauffer, 1945, for references).

The two chief fractions were (1) the 'Ba-insoluble fraction' precipitated at pH 8·2 by the addition of barium acetate, and (2) the 'Ba-soluble alcohol-precipitable fraction' formed after the removal of the Ba-insoluble material and the addition of ethyl alcohol to 75 %. The phosphate remaining in solution after the addition of alcohol will be termed the 'Ba-soluble alcohol-soluble fraction'. After removing barium with appropriate amounts of Na_2SO_4, the phosphates were fractionated further as (1) 'P inorganic', the true inorganic phosphate estimated with the reagents of Fiske & Subbarow (1925) after precipitation as $MgNH_4PO_4$ (Lohmann, 1928); 'P_0', phosphorus determined as phosphate which reacted directly with molybdate; 'P_7', 'P_{10}', 'P_{30}' and 'P_{180}', the phosphorus which appeared as orthophosphate after 7, 10, 30 and 180 min. hydrolysis in 1 N-HCl at 100° C. Some tissues of *Ascaris lumbricoides* contained substances which interfered with the direct estimation of phosphate. In such cases the phosphate was precipitated and washed before estimation. The total phosphorus, '$P_{tot.}$', was determined by the method of King (1932).

Estimations of the following substances were carried out according to the references given: fructose (Roe, 1934); glycogen (Good, Kramer & Somogyi, 1933); reducing value (Hagedorn & Jensen, 1923); lactic acid (Friedemann & Kendall, 1929); pentose (McRary & Slattery, 1945); and arginine (Macpherson, 1942). Nucleotide purine nitrogen was determined by the method of Kerr (1940). Adenosine triphosphate (A.T.P.) fractions were examined by means of frog muscle adenyl-deaminase (Parnas & Lutwak-Mann, 1935) the ammonia formed being distilled in the Parnas-Heller apparatus.

Gas changes were determined manometrically in Warburg vessels with low constants (Rogers, 1948).

The muscle adenylic acid and fructose 1:6-diphosphate used were commercial products of high purity. Coenzyme I was prepared by the method given by Williamson & Green (1940); Cori ester was prepared and used as the potassium salt (Hanes, 1940); glycogen was obtained from *A. lumbricoides* (Baldwin & King, 1942); A.T.P. was prepared from rabbit muscle (Umbreit, *et al.* 1945).

The extraction and estimation of the phospholipids

Two methods of extraction were used, one to obtain the total phospholipid and the other to extract cephalin with a minimum contamination of interfering nitrogenous substances. In the former case, known amounts of tissue (usually 7–8 g. or 20 ml. of freeze-dried perienteric fluid) were ground in a chilled mortar, dehydrated and extracted, first with cold 98% ethyl alcohol, then twice with hot 95% alcohol, and then continuously, over a period of 2 hr. with two portions of ethanol-ethyl ether (3:1, parts by volume) followed by extraction for 1 hr. with ether alone. The combined extracts were concentrated to about 1 ml. under reduced pressure at 50–55° C. The phospholipids were then taken up in five small portions of warm petroleum ether. To prevent the oxidation of lecithin, the extracted phospholipids were kept under carbon dioxide whenever possible.

The method of re-extraction with petroleum ether has been supported by many authors and criticized by others in that not all the phospholipid is extracted with this solvent. In the present work no chloroform-soluble phosphorus remained after treatment with petroleum ether except when perienteric fluid was used.

The petroleum ether extract was concentrated at reduced pressure and the phospholipids precipitated by acetone and magnesium chloride (Taurog, Entenman & Chaikoff, 1944). After washing with acetone, the precipitate was taken up in a mixture of ethyl ether and methyl alcohol. From this solution samples were taken for the determination of total phospholipid phosphorus, sphingomyelin, and total phospholipid choline from lecithin and sphingomyelin.

For the determination of cephalin, dehydration and extraction of the tissue was carried out with ethyl alcohol. Alcohol was removed from this extract under reduced pressure at 45° C., and the residue was extracted according to the method of Artom (1945*a*) to give the material for hydrolysis and subsequent serine and ethanolamine determination.

A small sample (usually 1/25th) of the methanol-ether solution mentioned above was dried, ashed and the $P_{tot.}$ determined (King, 1932). Lipid phosphorus × 25·5 was considered to give the total phospholipid.

To determine sphingomyelin, a sample (usually 2/5th) of the methanol-ether solution was dried and repeatedly extracted with small portions of hot methanol. Sphingomyelin was then precipitated from this solution as the reineckate (Marenzi & Cardini, 1943*a*). After washing with methanol, acetone and ether, the phosphorus in the pale pink sphingomyelin reineckate was determined (King, 1932). The calculation was carried out according to the following equation (Marenzi & Cardini, 1943*a*):

$$\text{phosphorus} \times 26{\cdot}84 = \text{sphingomyelin}.$$

The hydrolysis of the phospholipids with 5N-hydrochloric acid in methanol by the method of Thannhauser, Benotti & Reinstein (1939) modified according to Marenzi & Cardini (1943*a*) gave very low choline/phosphorus ratios compared with results obtained using barium hydroxide hydrolysis (Entenman, Taurog & Chaikoff, 1944). The following procedure for the estimation of the total phospholipid choline was therefore adopted:

To a 5 ml. sample of the methanol-ether solution 15 ml. of saturated barium hydroxide solution were added and the mixture heated on a boiling water-bath for about 2 hr. or till it was almost dry. The hydrolysed material was then taken up with hydrochloric acid, brought to about 1·2N, and repeatedly extracted with petroleum ether to remove fatty acids. Choline was precipitated as the reineckate in 1·2N-hydrochloric acid (Entenman *et al.* 1944) and estimated colorimetrically using standards prepared by the method of Marenzi & Cardini (1943*b*).

The total choline-containing phospholipid (lecithin and sphingomyelin) was calculated from the equation:

$$\text{choline} \times 6{\cdot}7 = \text{choline phospholipid}$$

(Marenzi & Cardini, 1943*a*).

After hydrolysing the cephalin fraction with 6N-hydrochloric acid in methyl alcohol, the solvent and acid were removed under reduced pressure and the hydrolysate taken up in water. Fatty acids were extracted with petroleum ether, the last traces of which were removed in a stream of air. Samples were taken for the determination of total serine plus ethanolamine and for the estimation of the individual components after separation on a permutit column.

Before use, the permutit was washed with 30% sodium chloride and distilled water until washings were chloride-free. The air-dried permutit was then set up in columns and the serine and ethanolamine separated by the method of Artom (1945*a*). Estimations were carried out at pH 9·5 with periodic acid (Artom, 1945*a*) in the Markham steam distillation apparatus, the ammonia being collected in saturated boric acid solution and titrated. Tests on the technique showed an accuracy within 4%. The

separation of known quantities of serine and ethanolamine reached this accuracy when a slow flow of the solution was passed through a 9 cm. column of permutit. A somewhat longer column than that used by Artom (1945*a*) was necessary, the permutit being rather coarse-grained.

Calculations (Artom, 1945*b*) were carried out as follows: serine-containing phospholipid = ml. 0·01 N-HCl × 7·90, ethanolamine-containing phospholipid = ml. 0·01 N-HCl × 7·50. A third factor, which depended on the proportion of serine and ethanolamine present in the particular tissue examined, was used for calculating the total cephalin.

PROCEDURE AND RESULTS

The utilization of exogenous carbohydrate by intact parasites

The respiratory activities of *Neoaplectana glaseri*, *Ascaridia galli*, *Nematodirus* spp. and *Nippostrongylus muris* were determined in small Warburg vessels (Rogers, 1948). With a gas phase of air, the addition of glucose to the medium increased the Q_{O_2} of *Neoaplectana glaseri* slightly, though there was a more marked rise in the Q_{CO_2}. Thus an R.Q. of 0·6 was raised to 1·0 over a period of 40 min. when 2 mg. glucose per ml. were included in the saline-phosphate buffered at pH 6·5 used as a medium (see Table 1). A small rise in the R.Q. was noted when *Nippostrongylus muris* was examined but no appreciable changes were obtained with *Ascaridia galli* or *Nematodirus* spp.

Table 1. *The effect of glucose on the respiration of* Neoaplectana glaseri

The oxygen uptake and R.Q.'s for 10 min. periods taken for 1 hr. are shown.

	Oxygen uptake, 1 μl. per mg. dry tissue		Respiratory quotient	
Time (min.)	Control	Glucose	Control	Glucose
0–10	2·17	2·03	0·55	0·56
10–20	2·05	2·07	0·52	0·82
20–30	2·02	1·81	0·55	0·99
30–40	1·97	1·94	0·57	1·03
40–50	2·10	1·90	0·59	1·05
50–60	2·03	1·97	0·59	1·10

Although small amounts of glucose disappeared from the medium in which *Neoaplectana glaseri* was examined, less than 1 % could be attributed to lactate formation, which was always small under aerobic conditions, and less than 40 % to increased CO_2 production. Glucose utilization, though still small, was greater when bicarbonate—CO_2 buffer (gas phase, 5 % CO_2 in air) was used instead of phosphate.

The addition of pyruvate to the medium in the Warburg vessels caused even smaller changes in respiratory activity than glucose.

It should be noted that bacterial contamination was least when *N. glaseri*, which had been grown under sterile conditions, was used. It is probable, therefore, that the changes in respiratory activity and glucose concentrations, though small, were due to the parasites rather than contaminating organisms. The failure of exogenous glucose to affect the respiration of complex organisms in which glucose uptake was probably dependent on feeding activity, and which usually contained large glycogen reserves, is not surprising.

The formation of ester phosphate

(*a*) *Muscle.* With glyoxaline buffer (Kirby & Neuberger, 1938) or low concentrations of phosphate buffer at pH 7·2, *Ascaris lumbricoides* muscle brei showed an increase in P_o and a decrease in ester phosphate during incubation. Water extracts prepared from unautolysed muscle, or brei buffered with higher concentrations (0·05 M) of phosphate showed a decrease in P_o and an increase in ester phosphate. These changes were more marked when fluoride was added. The results obtained when muscle brei was incubated in phosphate buffer, 0·05 M at pH 7·2, 0·002 M-adenylic acid, 0·005 M-$MgSO_4$, 0·2 % glycogen, toluene, with and without NaF, 0·2 %, are shown in Figs. 1 and 2.

The nature of the phosphorus compounds formed during incubation is suggested by the hydrolysis curves obtained with 1 N-HCl at 100° C. (see Fig. 3). There was a steady increase in both easily and difficultly hydrolysable phosphate compounds: the rate of increase was accelerated in the presence of fluoride.

It would appear that *A. lumbricoides* muscle contained phosphatase active at pH 7·2, which was not extracted from unautolysed tissue by water. The phosphatase was inhibited by high phosphate concentrations and by fluoride. Compounds which hydrolysed like A.T.P. and glucose 6-phosphate were formed when preparations, in which phosphatase was absent or inhibited, were incubated.

(*b*) *Intestine.* Brei prepared from *A. lumbricoides* intestine showed a rapid increase in P_o even when incubated in the presence of strong phosphate buffer. Sodium fluoride slightly inhibited P_o formation. The increase in P_o was accompanied by a decrease in acid-soluble and residual phosphorus compounds. The fall in acid-soluble phosphate was decreased when glucose was added to the brei.

The changes in the phosphorus compounds of the intestines of whole *A. lumbricoides* maintained *in vitro* were followed for 30 hr. Under anaerobic conditions, $P_{tot.}$, P_o and $P_{residual}$ fell markedly while the acid-soluble phosphate remained fairly constant.

Under aerobic conditions P_o fell slightly but the other components rose considerably.

(*c*) *Ovary.* The phosphorylative activity of brei prepared from the fine convoluted tubules of the ovary was greater than that of muscular tissue. Hydrolysis curves suggested the formation of a higher proportion of easily hydrolysable phosphate compounds in the ovary during incubation.

(*d*) *Perienteric fluid.* Periods of incubation up to 2 hr. did not cause any change in the P_o of perienteric fluid.

It would appear that, with the exception of the perienteric fluid, the chief tissues of *A. lumbricoides* contain active phosphatases and phosphorylating systems. Ovarian tissues were the most active.

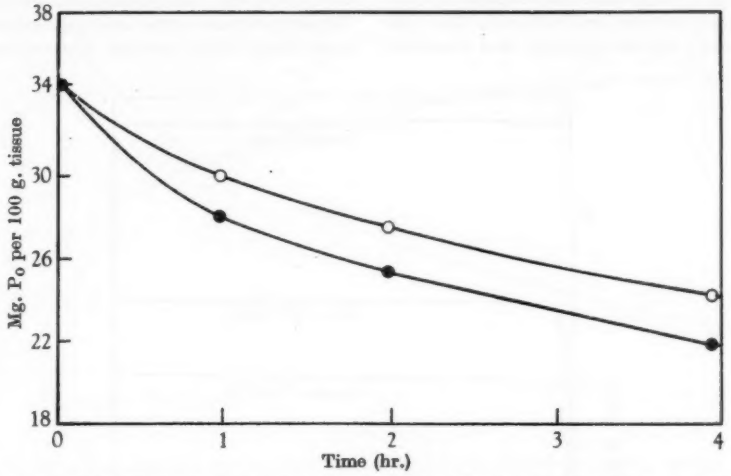

Fig. 1. The fall of P_o in *Ascaris lumbricoides* muscle brei incubated with (dots) and without (circles) fluoride. For further details, see text.

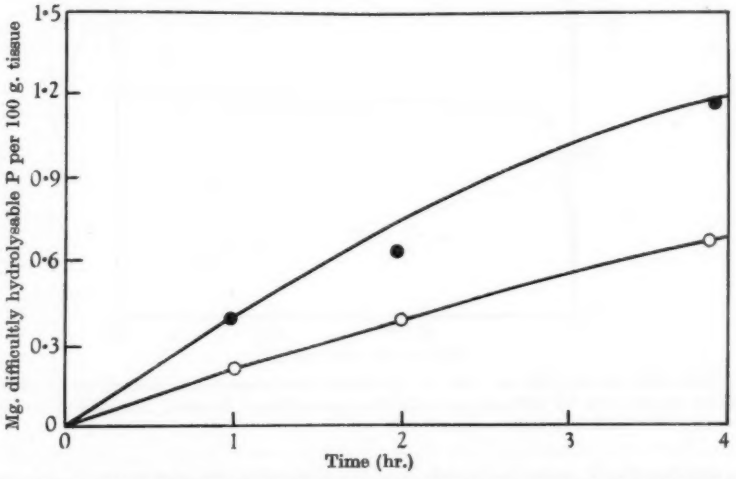

Fig. 2. The formation of difficultly hydrolysable phosphorus ($P_{tot.} - P_{120}$) in *Ascaris lumbricoides* muscle brei incubated with (dots) and without (circles) fluoride. For further details, see text.

The breakdown and synthesis of glycogen

Aqueous extracts of *Ascaridia galli* were used for these experiments. Perienteric fluid, which contains an active amylase, was removed, the tissues of the parasite were ground in water and allowed to autolyse at room temperature for 20–30 min. The water extract, separated by centrifuging, was incubated at pH 6·5. Adenylic acid, 1 mg./ml., and glucose 1-phosphate, 5 mg./ml., were added. At intervals during incubation samples were taken and glycogen, P_0 and P_{10} determined. The P_{10} fell rapidly during the first 15 min. incubation and thereafter remained fairly constant. The other results are shown in Fig. 5. Clearly polysaccharide was synthesized early in the experiment while glucose 1-phosphate was being

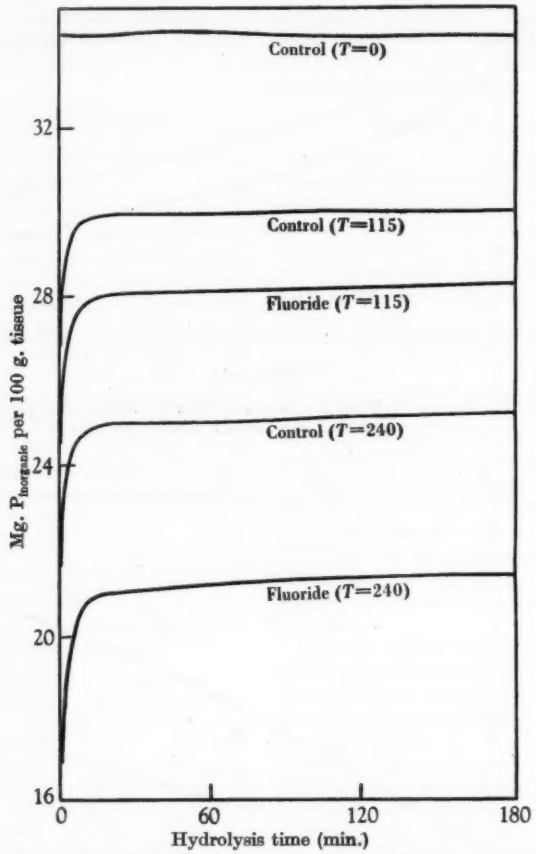

Fig. 3. The hydrolysis, in 1 N-HCl at 100° C., of phosphorus compounds formed by incubating *Ascaris lumbricoides* muscle brei for different periods with and without fluoride. The time of incubation (T) is given in min. For further details, see text.

at pH 7·2 and the changes in glycogen and glucose concentration determined at intervals. The results obtained are shown in Fig. 4. In 0·005 M-NaF the rate of disappearance of glycogen was decreased and smaller amounts of glucose were formed.

To obtain polysaccharide synthesis the water extract from unautolysed *A. galli* tissues was incubated in 0·005 M-NaF and buffered with phosphate metabolized. The fall in Cori ester phosphate after 15 min. incubation was probably masked by the formation of other easily hydrolysable compounds.

The formation of glycogen from Cori ester is a balance between the reactions

(1) Cori ester $\rightleftharpoons$ glycogen + phosphate,

and (2) Cori ester $\rightleftharpoons$ Embden ester.

In the presence of fluoride, glucose formation direct

from Cori ester was inhibited and reaction (1) proceeded rapidly but the equilibrium was continually disturbed by the transformation of the glucose 1-phosphate into Embden ester and ultimately re-

of a polysaccharide. It appears then, that a reaction of the type

glycogen + phosphate ⇌ glucose 1-phosphate

occurred in the tissues of *Ascaridia galli*, and was

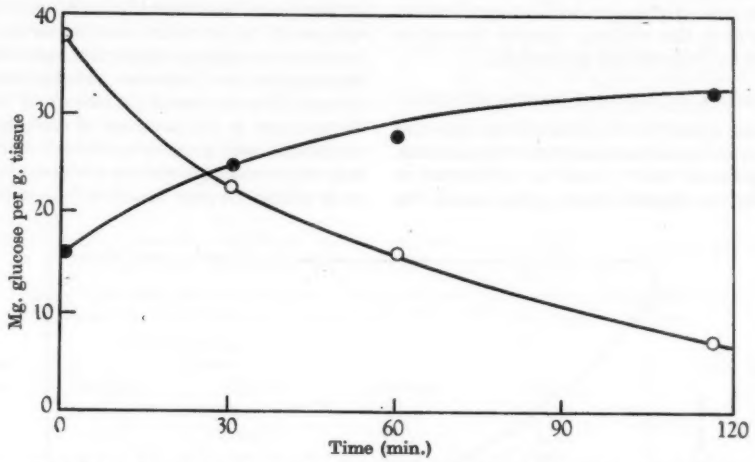

Fig. 4. The fall in glycogen (circles) and increase in glucose (dots) in incubated aqueous extracts of *Ascaridia galli*. For further explanation, see text.

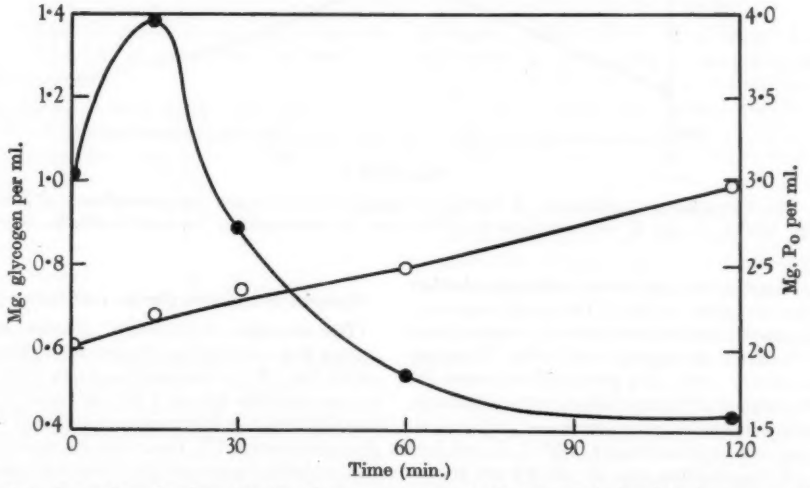

Fig. 5. The formation of polysaccharide (dots) and the rise in P_O (circles) in incubated aqueous extracts of *Ascaridia galli* after the addition of Cori ester. For further details, see text.

action (2) went to completion with a succeeding withdrawal of glycogen.

The identification of the 'glycogen' synthesized from Cori ester during the experiment depends only on the method used for the estimation. This can be considered as sufficient evidence for the formation

reversible under conditions similar to those used for the study of the system in mammalian tissues.

The synthesis of polysaccharide was demonstrated with brei prepared from *A. lumbricoides* muscle poisoned with fluoride but without the addition of Cori ester to the system. Activity was very low,

however, and long periods of incubation were necessary. With toluene and 0·005 M-NaF there was an increase in 'glycogen' for 3 hr. after which the amount present fell, reaching the initial level after a total incubation period of 6 hr. Phloridzin, 0·005 M, decreased the rate of glycogen utilization to about half that found in the control. Glucose formation was inhibited by fluoride and phloridzin.

The utilization and formation of fructose phosphates

The aqueous extracts of *Nematodirus* spp. and *Ascaridia galli* used in these experiments were almost free of compounds which could be estimated as fructose by the colorimetric resorcinol method. The Harden-Young ester. The increase in fructose at pH 7·2, however, may have been partly or wholly due to the accumulation of Neuberg ester or other fructose compounds. It appears then that fructose 1:6-diphosphate was catabolized by systems present in the parasites *Ascaridia galli* and *Nematodirus* spp., and, under other circumstances, fructose or fructose-containing compounds, probably fructose 6-phosphate or fructose 1:6-diphosphate, were formed. The increased utilization of fructose 1:6-diphosphate in the presence of diphosphopyridine-nucleotide and pyruvate, though slight, suggests that the hexosediphosphate took part in a glycolytic cycle similar to that found in mammalian tissues.

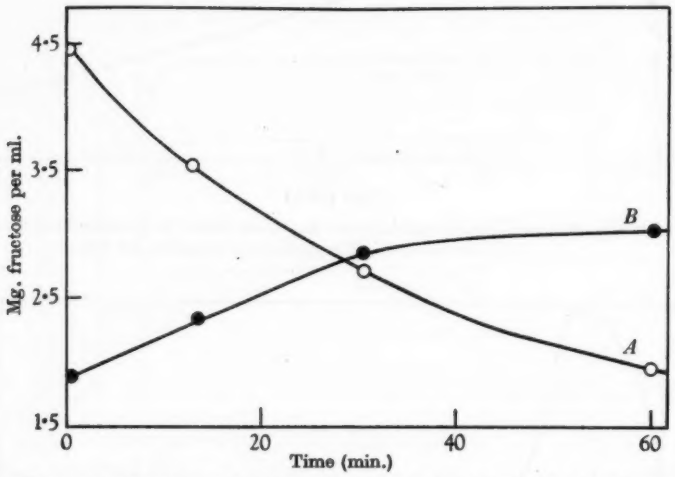

Fig. 6. The formation and utilization of fructose phosphates in incubated cell-free extracts of *Nematodirus* spp. Curves *A* and *B* were obtained at pH 6·8 and 7·2 respectively. For further details, see text.

changes in fructose concentration during incubation were followed after adding hexosediphosphate. Hexosediphosphate decreased when incubated with extracts buffered at slightly acid pHs. Fructose increased slowly when the preparations were incubated in neutral or slightly alkaline preparations.

The results obtained when fructose 1:6-diphosphate, 5 mg./ml., was incubated at 37° C. in cell-free extracts of *Nematodirus* spp. at pH 6·8 are shown in Fig. 6, curve *A*. The increase in fructose in cell-free extracts of *Nematodirus* spp. buffered at pH 7·2 after the addition of hexosediphosphate, 2 mg./ml., is shown in Fig. 6, curve *B*. The rate of utilization of fructose 1:6-diphosphate at pH 6·8 was increased slightly by adding coenzyme 1 and pyruvate.

The results, showing the fall in fructose after the addition of larger amounts of the diphosphate, may be taken as indicating the rate of utilization of the

Glycogen utilization, glucose and lactate formation

The changes in glycogen, glucose and lactate during the incubation of aqueous extracts of brei which had been autolysed for short (about 15 min.) or long periods (about 2 hr.) at room temperature were determined. Extracts were buffered with 0·05 M-phosphate at pH 7·3. On occasions, complete balance sheets could not be obtained from material prepared from the one lot of parasites. In such cases lactate changes were determined in similar but separate preparations. Results are shown in Tables 2 and 3.

Glucose formation in all preparations except that from *Nematodirus* tissue which had been autolysed for long periods, accounted for 55–60 % of the glycogen utilized during 1 hr. When extracts of *Nematodirus* brei which had been autolysed for long periods were used, the glucose formed was very much

greater than the glycogen decrease. Similar results were obtained with preparations from *Ascaridia galli* which had been autolysed for long periods when the perienteric fluid, which contained amylase, was not removed. It was impossible to remove perienteric fluid adequately from the small *Nematodirus* spp. It is considered that the excess glucose was due to the breakdown of dextrins, which were not estimated as glycogen, and which were formed by the action of perienteric-fluid amylase during the long autolysis period.

In preparations from tissues which had been autolysed for short periods, lactate rose during incubation (see Table 3). The increases, however, were small and there was a wide disparity in the glycogen-glucose-lactate balance sheet. In preparations from tissues which had been autolysed for long periods lactate fell during incubation. It would appear that during long periods of autolysis the system concerned with lactate formation lost activity more rapidly than the system utilizing lactate. This subject is being examined further in relation to pyruvate metabolism.

Table 2. *The formation of glucose and the utilization of glycogen in incubated extracts of parasite brei which had been autolysed for different periods.*

Perienteric fluid was removed from *Ascaridia galli* before the preparation of brei. Results are given in mg./g. tissue used. For further explanation, see text.

Incubation time (min.)	*Nematodirus* spp. Glycogen	*Nematodirus* spp. Glucose	*Ascaridia galli* Glycogen	*Ascaridia galli* Glucose	Autolysis time (min.)
Zero	9·6	2·5	34·0	22·9	15 or less
60	4·3	6·1	28·5	26·1	
120	—	—	22·1	29·7	
Zero	7·3	0·9	18·5	12·2	120 or more
60	3·8	8·9	10·8	17·1	
120	—	—	7·2	17·75	

Table 3. *Changes in lactate in incubated aqueous extracts of parasite brei which had been autolysed for different periods.*

Results are given in mg./g. tissue used. For further explanation, see text.

Incubation time (min.)	*Nematodirus* spp.	*Ascaridia galli*	Autolysis time (min.)
Zero	0·08	0·25	15 or less
60	0·18	0·37	
120	—	0·70	
Zero	0·16	1·00	120 or more
60	0·14	0·55	
120	—	0·48	

Acid production and bicarbonate utilization in cell-free extracts

Aqueous extracts of *Nematodirus* spp. and *Ascaridia galli*, buffered with CO_2-bicarbonate, were examined manometrically in a gas phase of oxygen-free 5% CO_2-nitrogen in an attempt to follow anaerobic acid production. However, bicarbonate in excess of that freed as gaseous CO_2 during the experiment was removed from the medium and prevented the manometric determination of acid formation.

The phosphorus compounds in the resting tissues of Ascaris lumbricoides

The phosphorus compounds have been grouped as (*a*) $P_{acid\text{-}sol.}$, (*b*) phospholipids, and (*c*) the remaining 'residual' phosphorus compounds. The percentage occurrence of these three fractions in the intestine, ovary, muscle and perienteric fluid of the parasite is shown in Table 4, where each figure represents the mean value of some three determinations. In contrast to the other tissues, muscle contained only acid-soluble phosphorus compounds. The high proportion of residual phosphorus in the ovary may have some relation to the very high egg production of *A. lumbricoides*.

Table 4. *The percentage occurrence of acid-soluble phosphorus compounds, phospholipids and residual phosphorus compounds in the tissues of* Ascaris lumbricoides.

	Intestine	Ovary	Muscle	Perienteric fluid
$P_{acid\text{-}sol.}$	45	26	98	74
Phospholipid P	33	39	—	21
Residual P	22	35	—	—

(1) *The acid-soluble phosphorus compounds.* Figures showing the hydrolysis of the $P_{acid\text{-}sol.}$ fractions in 1N-HCl at 100° C. are given in Table 5. The intestine showed a relatively high proportion of P_0 and easily hydrolysable compounds. Difficultly hydrolysable phosphorus compounds predominated in muscle.

Alkali-unstable phosphorus compounds in the parasites' tissues were small in amount. Inorganic pyrophosphate was absent.

The proportion of the $P_{acid\text{-}sol.}$ fraction precipitated as barium salts at pH 8·2 varied from 61 to 74 % in the intestine, 28 to 37 % in perienteric

fluid, 47 to 52 % in the ovary, and 21 to 34 % for muscle. Hydrolysis of the phosphorus compounds prepared from the Ba-insoluble fraction indicated that substances similar to A.T.P. were present. The amounts of A.T.P.-P_7 obtained were: perienteric fluid 1·14 mg./100 ml.; intestine 11·9 mg./100 g. tissue; ovary 8·2 mg./100 g. tissue; muscle 8·9 mg./100 g. tissue. After subtracting the amounts of P_0 present, $P_7/P_{tot.}$ in the Ba-insoluble fraction from the intestine varied from 2/3·9 to 2/5·0. The A.T.P.-amino-nitrogen freed by frog muscle deaminase was equivalent to an A.T.P.-$P_{tot.}$ of 10·6–12·7 mg./100 g. tissue (see Table 5).

Table 5. *The hydrolysis of acid-soluble phosphorus compounds from the tissues of* Ascaris lumbricoides *in* 1 N-HCl *at* 100° C.

Results are given in mg. P/100 g. tissue or per 100 ml. perienteric fluid. For further explanation, see text.

	Intestine	Ovary	Muscle	Perienteric fluid
P_0	41	11·2	18·2	3·1
P_7	66	17·6	32·2	4·2
P_{30}	—	18·4	37·5	4·7
P_{120}	71	21·3	40·4	5·8
P_{180}	73	22·3	43·3	6·5
$P_{tot.}$	105	46·5	102	18·9

were present in the tissues examined; but determinations based on the P_7 in Ba-insoluble material probably gave somewhat high results. Nucleotides other than A.T.P. were present in the ovary and perienteric fluid.

The phosphorus in the Ba-soluble 10 % alcohol-precipitable fractions formed less than 10 % of the total $P_{acid\text{-}sol.}$ Fructose was found in this fraction on two occasions only, when ovary and muscle were used.

The Ba-soluble 75 % alcohol-precipitable material formed 57–68 % of the total acid-soluble phosphorus compounds of muscle, 39–48 % in perienteric fluid, 17–31 % in the ovary, and 8·9–12·5 % in the intestine. Hydrolysis of the phosphorus compounds prepared from these fractions in 1 N-HCl showed the presence of very small amounts of acid-unstable compounds and large amounts of difficultly hydrolysable material. Inorganic phosphate was absent or present in traces only.

The acid-unstable fraction, when hydrolysed for 15 hr. in 0·1 N-HCl at 30° C., gave 0·9 mg. P/100 ml. perienteric fluid and 1·2 mg. P/100 g. muscle. The rate of hydrolysis was not increased by molybdate. Samples taken after hydrolysis were used for arginine estimation (Macpherson, 1942). Characteristic colours indicating 0·8 mg. arginine/100 ml. perienteric fluid and 1·2 mg./100 g. muscle were obtained. The colours faded rapidly, however, and estimations were repeated using phosphagen fractions which had been purified as follows: The Ba-soluble 75 % alcohol-precipitable fraction was prepared and the barium removed with a minimum amount of Na_2SO_4. After hydrolysing for 15 hr. in 0·1 N-HCl, the unhydrolysed phosphorus compounds were reprecipitated with barium acetate. The hydrolysate contained small amounts of $P_{inorganic}$ but failed to give a Sakaguchi reaction. The presence of arginine in suitably prepared extracts was examined with arginine decarboxylase from *Escherichia coli*. Very small amounts of CO_2 were produced and it was considered that arginine was absent or present in very small amounts only.

Table 6. *The analysis of the Ba-insoluble fraction from three samples of* Ascaris lumbricoides *intestine.*

P_7 and $P_{tot.}$ figures apply to organic phosphorus compounds only. Results are given in mg./100 g. tissue. For further explanation, see text.

Nucleotide purine N	Equivalent A.T.P.-P	A.T.P.-amino N	Equivalent A.T.P.-P	P_7	$P_7/P_{tot.}$
10·1	13·4	1·92	12·7	14·5	2 : 3·9
14·8	19·6	1·60	10·6	10·4	2 : 5·0
12·6	16·7	1·71	11·4	10·7	2 : 4·4

The Ba-insoluble fraction from the ovary gave $P_7/P_{tot.}$ ratios varying from 2/3·6 to 2/4·4. On the basis of A.T.P.-P_7 figures, the A.T.P.-NH_2-N recovered in two experiments was 76·2 and 77·6 %. High $P_7/P_{tot.}$ ratios were obtained for perienteric fluid where the P_7/pentose ratio was in the region of 1/2·4. The amounts of easily hydrolysable phosphorus compounds in the perienteric fluid were too small to be estimated with frog muscle deaminase.

The occurrence of substances similar to A.T.P. in the tissues of *A. lumbricoides* was examined further by estimating nucleotides in the acid-soluble material. Nucleotide purine nitrogen found in three different samples of intestinal tissue is shown in Table 6. The Ba-precipitable material obtained from intestinal tissue after the removal of nucleotides contained very little easily hydrolysable phosphate. Nucleotide purine nitrogen in ovary and perienteric fluid indicated the presence of nucleotide in excess of that calculated from P_7 figures.

It is suggested that A.T.P. or similar compounds

After the hydrolysis of the acid-unstable material, the Ba-soluble 75 % alcohol-precipitable fraction from muscle gave a hydrolysis curve similar to that of Embden ester. A sample which had been freed of acid-unstable material and precipitated with barium

and alcohol was washed with alcohol and ether and dried in a desiccator. The phosphorus content was 7·3%; the ratio of reduction value (Hagedorn & Jensen, 1923) to phosphorus content was 3·5.

The hydrolysis of the Ba-soluble 75% alcohol-precipitable fraction from ovary was somewhat similar to that from muscle, but the fraction recovered from perienteric fluid hydrolysed only 2·1–3·0% in 120 min., and 3·5–5·2% in 180 min. Further, fructose was absent from this latter material, the reducing value was equivalent to 9·4 mg. glucose/100 ml., the pentose value, 8·7 mg./100 ml., and the $P_{tot.}$, 6·6 mg./100 ml. Clearly, Neuberg ester was absent and Robison ester was either absent or present in small amounts only.

The phosphorus compounds which were not precipitated as barium salts in 75% alcohol were not examined.

(2) *The phospholipids.* The results obtained from the analyses of two samples of intestine, ovary and perienteric fluid are shown in Table 7. Phospholipid phosphorus in muscle formed less than 2% of the $P_{tot.}$ and analysis was not attempted. Total phospholipid was obtained by calculation from the total phospholipid phosphorus found; lecithin was calculated from the figures for total choline phospholipid and sphingomyelin, and cephalin was obtained by subtracting the total choline phospholipid from the total phospholipid.

Figures for cephalin determined by analysis and the amounts of serine- and ethanolamine-containing phospholipid found in two samples of ovary, intestine and perienteric fluid are shown in Table 7. The 'Total cephalin' was calculated as the sum of the serine- and ethanolamine-containing phospholipids. The 'Total cephalin found', determined as the ammonia from the cephalin hydrolysate, was always higher than the 'Total cephalin', obtained by estimating ammonia from the serine and ethanolamine separated on the permutit column. This discrepancy was probably due to pigments which were freed during the hydrolysis of the cephalin fraction. Intestine and perienteric fluid gave yellow, and ovary, red pigments. Ammonia from these pigments would tend to give high serine-ethanolamine figures when the two substances were determined together without passage through the column. When serine and ethanolamine were estimated separately, however, a lower figure for the sum was obtained because the pigments were retained on permutit and were not eluted by strong NaCl solutions.

Sphingomyelin was low in all tissues and sphingosine would not interfere appreciably with cephalin estimations.

The values for cephalin shown in Table 7 are smaller than those found by analysis (Table 8). The disparity in the results may have been due to the presence of contaminating nitrogenous compounds, or 'cephalins' in the parasite may be different in structure to those from mammalian tissues.

Table 7. *Phospholipids in the tissues of* Ascaris lumbricoides

Results are given in mg./100 g. of tissue or 100 ml. of perienteric fluid. Total phospholipid, total choline-containing phospholipid and sphingomyelin were determined by analysis. Figures for lecithin and cephalin were obtained by calculation.

Tissue	Total phospholipid	Total choline-containing phospholipid	Molal ratio choline/P	Lecithin	Cephalin	Sphingomyelin
Intestine	1738	1085	0·61	1029	753	56
	1655	1079	0·60	1031	576	48
Perienteric fluid	200	141	0·68	122	59	19
	191	148	0·75	137	43	11
Ovary	1890	1160	0·60	1160	730	nil
	1956	1201	0·63	1201	755	nil

Table 8. *The analysis of the cephalin fraction from tissues of* Ascaris lumbricoides

Results are given in mg./100 g. of tissue or 100 ml. of perienteric fluid. 'Total cephalin' was determined by analysis: 'Total cephalin found' was calculated as the sum of the serine- and ethanolamine-containing phospholipids.

Tissue	Total cephalin	Ethanolamine-containing phospholipid	Serine-containing phospholipid	Total cephalin found
Intestine	1150	641	234	875
	1351	655	269	924
Perienteric fluid	237	128	24	152
	227	119	18	137
Ovary	812	501	216	717
	823	487	196	683

DISCUSSION

The results obtained in the present investigation suggest that glycolysis of the type found in mammalian muscle occurs in a variety of nematode parasites. Glycogen was rapidly catabolized and lactate appeared. In addition, a rapid formation of glucose from glycogen was observed. In preparations which were autolysed for long periods the rate of removal of lactate exceeded the rate of formation. The utilization of Cori, Embden and Harden-Young esters and the actions of inhibitors on the parasite preparations was in accordance with the cycle of glycogen fermentation in yeast and mammalian muscle. *Ascaris lumbricoides* muscle differed from mammalian muscle (for references see Cori, 1941) in the ease with which polysaccharide formation took place without the addition of glucose 1-phosphate. The disappearance of bicarbonate and the metabolism of lactate in incubated preparations is being further examined. The formation of easily hydrolysable phosphate in incubated preparations and the occurrence of compounds identical or similar to A.T.P. suggest that energy transferring mechanisms associated with glycolysis were of the type found in mammalian tissues. However, it appears that phosphagens of the accepted type (Baldwin, 1933) were absent from the *A. lumbricoides* tissues used in these experiments. That such labile substrates would survive for any length of time in parasites which had been removed from a highly specialized environment is doubtful. The comparison of acid-soluble P compounds in the mammalian intestine, determined from hydrolysis curves and barium fractionation (Verzár & McDougall, 1936; Eiler, Stockholm & Althausen, 1940; Beck, 1942) with those in *A. lumbricoides* intestine may be briefly summarized as follows: A.T.P.-P of the parasite was two to three times as great as in the mammal; acid-unstable P, though differing in nature, was present in similar amounts; the difficultly hydrolysable P in the parasite was about half that found in the mammal; $P_{inorganic}$ reached the same high level as in the mammal.

The 'cell-free' perienteric fluid differed widely from mammalian blood serum in composition (for references, see Guest & Rapoport, 1941) and may be regarded as an intracellular fluid on biochemical as well as anatomical grounds (Goldschmidt, 1906). The resemblance of the acid-soluble organic P compounds of perienteric fluid to those normally occurring in mammalian tissues was probably superficial though the 'A.T.P.' fraction formed insoluble barium salts, precipitated as nucleotide and gave an A.T.P.-like hydrolysis curve.

The phosphorus distribution in the muscle of the parasite may be compared with that in the fly, rat and frog (for references, see Baldwin & Needham, 1933). In *A. lumbricoides* A.T.P.-P was about one-third of that found in the insect or frog; hexose phosphate P varied from six to twelve times the amounts in the vertebrates and three times the amount in the insect; phosphagen P, if present in the parasite at all, was very much lower than in the other organisms; $P_{inorganic}$ was similar to that in frog muscle but somewhat less than that found in the fly. The low-resting values for hexosemonophosphate P in mammalian muscle were obtained only when the tissues were removed under anaesthesia (Cori & Cori, 1931–32) and any stimulation led to greatly increased values. It is possible, then, that the removal of the parasites to an abnormal environment which stimulated activity gave rise to the high values obtained for the Ba-soluble alcohol-precipitable ester P. The effect of transferring parasites from the host to a relatively highly oxygenated medium may have affected phosphate compounds other than those in muscle (see p. 304).

In comparing the phospholipid content of *A. lumbricoides* tissues with that of vertebrate animals it must be emphasized that the methods and conversion factors used in the present investigation had been designed for use with compounds from mammalian tissues. The resemblance of phospholipids from widely different organisms may be superficial only and the value of the results must be judged accordingly. Nevertheless, the present results may have a significance at least equal to that claimed for most phospholipid analyses where cephalin has usually been estimated by difference only.

The total phospholipid of the parasite intestine was similar in amount to that of the rat intestine (Sinclair, 1929), but somewhat higher than in the ox intestine. The diaminomonophosphatide content was less than half that of the ox intestine: the choline-containing monoaminomonophosphatide in the parasite was somewhat greater than in the ox intestine but was lower than that of the brain, liver or kidney (Kaucher, Galbraith, Button & Williams, 1943). The non-choline-containing monoaminomonophosphatide content was much higher than that in ox intestine, and was of the same order as in liver, kidney and lung.

The amount of phospholipid in the parasite muscle was lower than in mammalian muscle (Bloor, 1936). The perienteric fluid, both from the nature and amounts of phospholipid found, superficially resembled mammalian blood plasma. In comparison with mammalian visceral organs, the parasite ovary was notable for the lack of sphingomyelin and the relatively high cephalin content.

As reported by Artom (1945*b*) for mammalian tissues, the serine-containing phospholipid was small compared to the ethanolamine fraction.

The report that the choline-phosphorus molal ratio for plasma phospholipid is higher when barium

hydroxide hydrolysis is used instead of acid hydrolysis (Taurog *et al.* 1944) is supported by the present work, for hydrolysis with 5N-hydrochloric acid in methanol gave figures much lower than those shown in Table 7 which were obtained after barium hydroxide hydrolysis.

SUMMARY AND CONCLUSIONS

1. The R.Q. of whole *Neoaplectana glaseri* rose from 0·56 to 1·10 when glucose was added to the medium. The Q_{O_2} was only slightly affected. Respiration of other nematode parasites was largely unaffected by exogenous glucose. Pyruvate had little or no effect.

2. Inorganic phosphate increased when *Ascaris lumbricoides* muscle brei was incubated. In the presence of fluoride, or when aqueous extracts were used, easily and difficultly hydrolysable phosphate was formed. Phosphatase activity was dominant even in aqueous extracts of *A. lumbricoides* intestinal tissues and fluoride inhibited P_0 formation only slightly. $P_{tot.}$, and $P_{residual}$ fell markedly in whole *A. lumbricoides* which had been incubated anaerobically. Under aerobic conditions P_0 fell slightly, but the other components rose considerably. Phosphorylative activity of *A. lumbricoides* ovary was greater than in the muscle. Phosphorus compounds in perienteric fluid did not change during incubation.

3. The breakdown of glycogen and the formation of glucose in aqueous extracts of *Ascaridia galli* was inhibited by fluoride and phloridzin. Polysaccharide was synthesized in fluoride-poisoned preparations when glucose 1-phosphate was added. Polysaccharide formation in *Ascaris lumbricoides* fluoride-poisoned muscle preparations was demonstrated without the addition of Cori ester.

4. Fructose 1:6-diphosphate added to aqueous extracts of *Ascaridia galli* and *Nematodirus* spp. was rapidly metabolized at pH 6·5. At pH 7·2 the fructose content increased.

5. In aqueous extracts of *Nematodirus* or *Ascaridia* brei which had been autolysed for short periods, 55–60 % of the glycogen utilized appeared as glucose. Only small amounts of lactate were formed. Aqueous extracts of *Nematodirus* and *Ascaridia* brei which had been autolysed for long periods formed glucose in excess of the glycogen disappearing. When perienteric fluid, which contained amylase, was removed from *A. galli* before autolysis, the amount of glucose formed was very much reduced. It is suggested that the excess glucose was formed from dextrins which were not estimated as glycogen and which were hydrolysed by perienteric fluid amylase during incubation.

Lactate decreased during the incubation of extracts prepared from brei which had been autolysed for long periods.

6. Bicarbonate decreased during the anaerobic incubation of aqueous extracts of *Nematodirus* spp. and *Ascaridia galli* which were buffered with CO_2-bicarbonate. The decrease in bicarbonate was in excess of the CO_2 expelled from the medium by acid formation.

7. The acid-soluble phosphorus compounds of the different tissues of *Ascaris lumbricoides* were examined. Compounds similar to A.T.P. were found in the intestine, ovary, muscle and possibly also in the perienteric fluid. The acid-unstable phosphorus compounds did not appear to be either creatine- or arginine-phosphate. Compounds similar to Embden ester were found in the ovary and muscle. The occurrence of the phosphate compounds found in the parasite is compared with figures given for other organisms.

8. It is concluded that carbohydrate fermentation as far as lactate in nematode parasites was similar to that found in yeast and mammalian muscle. Energy was transferred by means of high-energy phosphate bonds.

9. Phospholipids, which were found in considerable amounts in the tissues of *A. lumbricoides*, were estimated as lecithin, sphingomyelin and serine- and ethanolamine-containing phospholipids. The amounts of the phospholipids found in the parasite tissues are compared with figures given for other organisms.

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HOST-PARASITE RELATIONSHIP BETWEEN THE AMERICAN STREBLIDAE AND THE BATS, WITH A NEW KEY TO THE AMERICAN GENERA AND A RECORD OF THE STREBLIDAE FROM TRINIDAD, BRITISH WEST INDIES (DIPTERA)

By B. JOBLING, F.R.E.S., *Wellcome Entomological Field Laboratories, Wellcome Laboratories of Tropical Medicine, London*

(With 3 Figures in the Text)

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INTRODUCTION

In 1945 I received a large collection of Streblidae from the late Prof. A. M. Adamson, of the Imperial College of Tropical Agriculture. This collection was made by a post-graduate student, Mr T. S. Jones, and Dr E. C. Humphries, in Trinidad. Later on Mr Jones sent me another three collections, which together with the first comprised three hundred and sixty specimens, the largest number of Streblidae which has ever been collected in America. In addition, I received seven specimens from Mr P. C. J. Brunet, of Keble College, Oxford, which were also collected in Trinidad. I take this opportunity to express my thanks to all these gentlemen, and especially to Mr Jones, who also supplied me with the list of the bats of Trinidad, and some useful information regarding their roosting habits. My thanks are also due to Dr T. C. S. Morrison-Scott and Mr R. W. Hayman of the British Museum (Natural History) for their help in checking the names of several species of bats. The present record is based on the above-mentioned five collections and also includes all those records which had been known from Trinidad before.

The section on the host-parasite relationship is chiefly compiled from the notes which were made from time to time during the study of the specimens in the collections, which were sent to me for identification, from America. In preparation of this part I was hampered by the lack of information on the life history of these flies. For instance, there is no information on the duration of the uterine life of the larva and the length of the pupal stage. It is also not known how long a newly emerged fly can survive without host. All these points need studying in the caves and other places which are frequented by bats, together with the numerical record of these flies and their distribution among their hosts. This sort of work may produce some information of general parasitological interest, and may throw some light on the relationship between the economically important ectoparasitic insects and their hosts.

The illustrations for a new Key to the American genera were made from the mounted specimens, except Fig. 3 B, G and H, of which the first is adapted from Curran (1935), and the last two from Dybas & Wenzel (1947).

One doubtful genus, *Megistopoda* Macquart, 1852, is not included in this Key. It has already been shown in my previous paper (1936) that Macquart's specimen was probably a Nycteribiid of the genus *Basilia*. I also suspect that *Aspidoptera busckii* Coquillett, 1899, is identical with *A. phyllostomatis* Perty, 1830. This is the only species of this genus in which the wings are (about) as long as the thorax and the tibiae are pubescent. Of these two characters the first was mentioned by Perty, and the second by Coquillett.

In Table 1 *Synthesiostrebla amorphochili* Townsend and *Speiseria ambigua* Kessel are included in the subfamily Trichobiinae. My recent re-examination of the structure of these two Streblids has shown that they do not belong to the Streblinae.

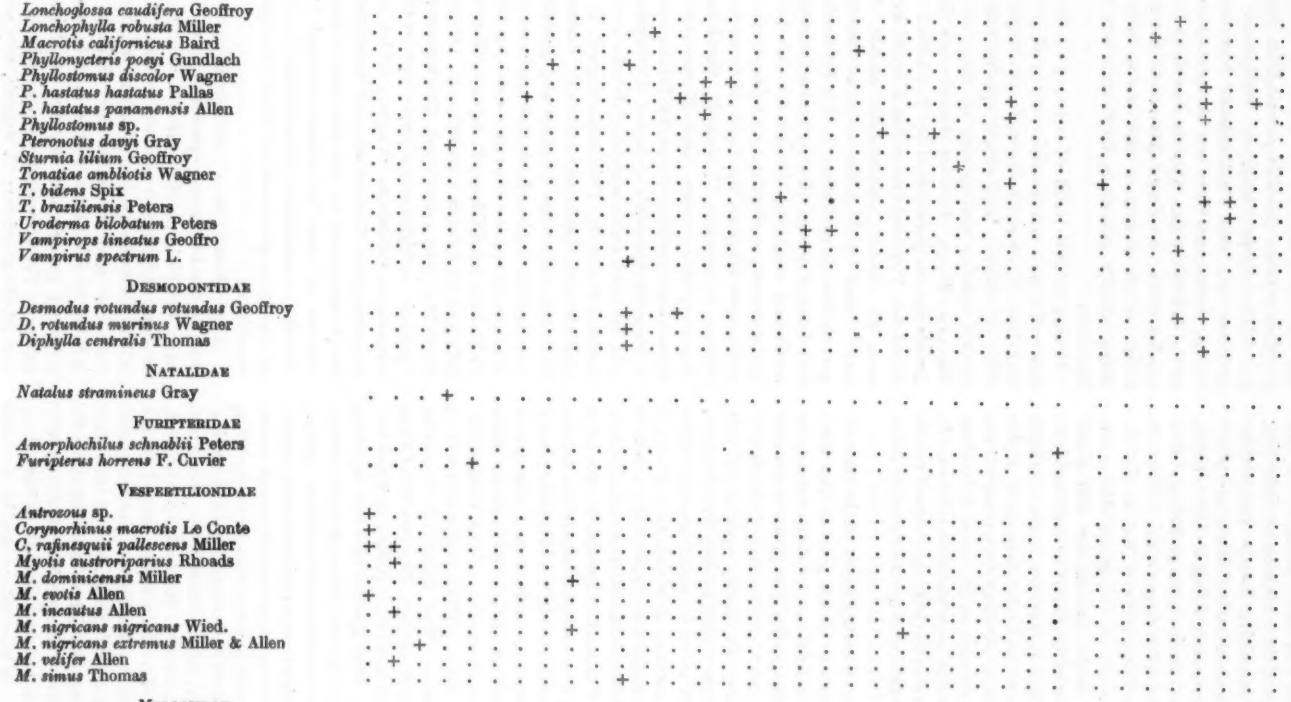
HOST-PARASITE RELATIONSHIP

The Streblidae of the New World form two distinct geographical groups: (1) the Nearctic and (2) the Neotropical. To the first group belong *Trichobius corynorhini* and *T. major*, which occur in the southern part of the Nearctic region; the first in the western

Table 1. *Showing the species of the American Streblidae and the species of bats which they parasitize*

The host of *Trichobius costalimae* and of *Strebla longirostris* is not known.

| Families, genera and species of bats | Sub-family Trichobiinae | | |


Lonchoglossa caudifera Geoffroy
Lonchophylla robusta Miller
Macrotis californicus Baird
Phyllonycteris poeyi Gundlach
Phyllostomus discolor Wagner
P. hastatus hastatus Pallas
P. hastatus panamensis Allen
Phyllostomus sp.
Pteronotus davyi Gray
Sturnia lilium Geoffroy
Tonatiae ambliotis Wagner
T. bidens Spix
T. braziliensis Peters
Uroderma bilobatum Peters
Vampirops lineatus Geoffro
Vampirus spectrum L.
DESMODONTIDAE
Desmodus rotundus rotundus Geoffroy
D. rotundus murinus Wagner
Diphylla centralis Thomas
NATALIDAE
Natalus stramineus Gray
FURIPTERIDAE
Amorphochilus schnablii Peters
Furipterus horrens F. Cuvier
VESPERTILIONIDAE
Antrozous sp.
Corynorhinus macrotis Le Conte
C. rafinesquii pallescens Miller
Myotis austroriparius Rhoads
M. dominicensis Miller
M. evotis Allen
M. incautus Allen
M. nigricans nigricans Wied.
M. nigricans extremus Miller & Allen
M. velifer Allen
M. simus Thomas
MOLOSSIDAE
Molossus obscurus Geoffroy
Tadarida antillularum Miller
T. bahamensis Rehn.

and the second in the eastern half (Jobling, 1938). These two species are the most primitive of the American Streblidae, and they occur on the following three genera of the primitive family Vespertilionidae: *Antrozous*, *Corynorhinus* and *Myotis*, of which the last-mentioned genus, according to Allen (1939), is the most ancient and also is the most widely distributed at present. *Trichobius corynorhini* is more restricted to the genus *Corynorhinus*, but in the extreme west it also occurs on the genus *Antrozous*, whereas *Trichobius major* parasitizes the genus *Myotis*. In those parts of the Nearctic region, where the distribution of their respective hosts overlaps, and where their hosts roost in the same cave, as, for instance, in Kansas, both of them may occur on the same species of bat and also on the same individual bat. This shows that the host-parasite relationship between these species and the bats is governed by the geographical distribution and by the roosting habit of the bats.

The influence of the roosting habit of the bats on these parasitic Diptera is better seen in the Neotropical group of the Streblidae, to which belong the rest of the New World species. The greatest number of these Streblidae occur on the bats of the family Phyllostomidae (Table 1). Many species of this family are common bats which usually live in caves, where some of them roost in small groups, while others form large bunches. A bunch or a group of one species may hang apart or quite close to that of another species, or the individuals of one species may roost among those of the other species. To the cave dwellers also belong the vampire bats of the family Desmodontidae; the fish-eating Noctilionidae; many Molossidae and Vespertilionidae; and the genus *Natalus* of the Natalidae, which all harbour these parasites. The genus *Desmodus*, of the Desmodontidae, according to Allen (1939), prefer to roost in fissures of large caves. The habit of *Desmodus rotundus rotundus* Geoffroy (syn. *D. rufus* Wied.) was described by Verteuil and Urich (1936), in Trinidad. According to them, this species has two roosting places: one where it digests the meal and another where it sleeps. The first may be a drain, bridge, stable or an uninhabited room; the second is usually a cave, hollow tree or a large underground drain. They have found the following bats in the roosting places of this species of *Desmodus*: *Carollia perspicillata perspicillata* L. (syn. *Hemiderma brevicauda* Wied.), *Artibeus planirostris* K. And., *Noctilio* sp., *Vampirus spectrum* L., *Enchistenes harti* Thomas, *Phyllostomus hastatus hastatus* Pallas, *Saccopteryx* sp. and *Micronycteris megalotis* Gray. On all these bats they have found Streblidae, but unfortunately did not publish the record of the species. Mr Jones, who has also studied this bat in Trinidad, informed me that it roosts singly or in small groups in the same roosting place as other bats, but not normally very close to them. In various roosting places he found this species with one or more of the following Phyllostomidae: *Carollia perspicillata perspicillata* L., *Chilonycteris rubiginosa rubiginosa* Wagner, *Artibeus* sp., and *Phyllostomus hastatus hastatus* Pallas. Sometimes there was also present one species of the Noctilionidae, *Noctilio leporinus* L. This last species roosts in narrow fissures of the rocks, tree holes, eaves of houses and caves, where individuals usually gather in large numbers and close together.

Owing to this habit of roosting in the confined space of a cave, many species of the Phyllostomidae and those of the genus *Desmodus* are readily infested with Streblidae, and single species may harbour several species and genera of these ectoparasitic Diptera. Thus, *Artibeus jamaicensis jamaicensis* Leach can be parasitized by *Trichobius mixtus*, *T. truncatus*, *T. pseudobruncatus*, *Paratrichobius longicrus*, *P. dunni*, *Nycterophilia coxata*, *Pterellipsis aranea* and by *Metelasmus pseudopterus*; *Carollia perspicillata perspicillata* L. by *Trichobius dugesii*, *T. mixtus*, *T. perspicillatus*, *Speiseria ambigua*, *Paratrichobius longicrus*, *Euctenodes mirabilis* and by *Metelasmus pseudopterus*; *Chilonycteris rubiginosa rubiginosa* Wagner, by *Trichobius coecus*, *T. parasiticus*, *T. sparsus*, *T. dugesii*, *Speiseria ambigua*, *Nyceterophilia coxata* and *Euctenodes mirabilis*; *Glossophaga soricina* Pallas, by *Trichobius uniformis*, *T. dugesii*, *Speiseria ambigua*, *Paradyschiria dubia* and by *Euctenodes mirabilis*; *Phyllostomus hastatus hastatus* Pallas, by *Trichobius dugesii*, *T. mixtus*, *T. phyllostomae*, *Aspidoptera minuta*, *Euctenodes mirabilis*, *Paraeuctenodes longipes* and probably also by *Aspidoptera clovisi*, *A. phyllostomatis* and *Pterellipsis aranea*; *Desmodus rotundus rotundus* Geoffroy, by *Trichobius parasiticus*, *T. dugesii*, *Euctenodes mirabilis* and by *Strebla vespertilionis*. Of the species of Streblidae just mentioned, *Trichobius dugesii* and *Speiseria ambigua* sometimes occur on *Chilonycteris rubiginosa rubiginosa* Wagner, which is not their principal host. The presence of these species on this bat is explained by the fact that sometimes individuals of this bat roost amongst numbers of *Carollia perspicillata perspicillata* L., which is one of the principal hosts of these two species of the Streblidae.

It has already been mentioned that a single cave may be used by many species of bats. In the same cave, in Trinidad, Mr Jones has found six different species belonging to as many genera. The tendency of the different species of bats to frequent the same cave has also influenced the adaptation of many species of Streblidae to the bats belonging to the different genera and families of Chiroptera. For example, *Trichobius mixtus* parasitizes four genera of the Phyllostomidae; *T. dugesii*, five genera of the Phyllostomidae and one genus of the Desmodontidae;

T. coecus, two genera of the Phyllostomidae and one genus of the Natalidae; *T. phyllostomae*, one genus of the Phyllostomidae and one genus of the Molossidae; *T. parasiticus*, three genera of the Phyllostomidae, two genera of the Desmodontidae and one genus of the Vespertilionidae; *Nycterophilia coxata*, four genera of the Phyllostomidae; and *Euctenodes mirabilis*, five genera of the Phyllostomidae and two genera of the Desmodontidae. Although each of the species just mentioned can parasitize several species of the cave bats, they do not occur on many other species, even if the latter use the same cave together with those parasitized. The freedom of these bats from these Streblidae is more probably due to their roosting far apart from the infested bats, or to the roosting of the individual bats some distance away from each other. It has already been observed by Phillips (1924) that the Rhinollophidae and the Emballonuridae of Ceylon are generally free from Streblidae. He attributes this to their habit of hanging apart from each other when at rest. There is no record of Streblidae from the American cave bat, *Mormoops megalophylla* Peters, although in the same cave, in Mexico, Ward (1904) has found this bat together with *Chilonycteris rubiginosa rubiginosa* Wagner, *Pteronotus davyi* Gray, *Natalus stramineus* Gray and *Myotis velifer* Allen, which are usually infested with these flies. The absence of the record of Streblidae on *Mormoops megalophylla* is more probably due to the lack of the examination of this bat for these parasites than to its roosting habit.

Among some heteroxenous Streblidae the host preference is still quite distinct, and it is even possible in some cases to determine their principal host. Thus, *Trichobius coecus* is much more common on *Chilonycteris rubiginosa rubiginosa* Wagner than on *Pteronotus davyi* Gray and *Natalus stramineus* Gray, to which undoubtedly it has adapted itself much later. The same can be said about *Trichobius parasiticus*, which is very common on the Desmodontidae especially on *Desmodus rotundus rotundus* Geoffroy, which should be considered as its principal host. Of the other heteroxenous Streblidae, *Nycterophilia coxata* is much more common on widely distributed *Chilonycteris rubiginosa rubiginosa* Wagner than on the other four species of bats shown in the table; *Speiseria ambigua*, on *Carollia perspicillata perspicillata* L.; and *Strebla vespertilionis*, on *Anoura geoffroyi geoffroyi* Gray. On the other hand, some other heteroxenous Streblidae have so completely adapted themselves to different species and genera of bats that it is impossible to establish their principal host. Thus, *Paratrichobius longicrus* parasitizes more or less equally *Carollia perspicillata perspicillata* L. and *Artibeus jamaicensis jamaicensis* Leach and its subspecies; *Trichobius dugesii* is much more common on *Glossophaga soricina* Pallas and *Carollia perspicillata perspicillata* L.; *Trichobius mixtus* occurs more frequently on species of the genus *Artibeus* and on those of the genus *Phyllostomus*, while *Euctenodes mirabilis* is more common on the Desmodontidae and in a slightly smaller degree on *Phyllostomus hastatus hastatus* Pallas and its subspecies.

As far as is known at present only nine species of the Streblidae are restricted each to a single species of bat. Thus, *Trichobius sparsus* Kessel is only known from *Chilonycteris rubiginosa rubiginosa* Wagner; *Trichobius hirsutulus* Bequaert, from *Myotis nigricans extremus* Miller; *Trichobius palidus* Curran, from *Furipterus horrens* Cuvier; *Trichobius sphaeronotus* Jobling, from *Leptonycteris nivalis* de Saussure; *Pseudostrebla riberio* Costa Lima, from *Tonatia amblyotis* Wagner; *Joblingia schmidti* Dybas & Wenzel, from *Myotis nigricans nigricans* Wied.; *Aspidoptera megastigma* Speiser, from *Noctilio leporinus* L.; *Synthesiostrebla amorphochili* Townsend, from *Amorphochilus schnablii* Peters; and *Eldunnia breviceps* Curran from *Lonchophylla robusta* Miller. Of these species *Aspidoptera megastigma* has been recorded many times, *Synthesiostrebla amorphochili* has two records, while each of the other species has been found only once and, therefore, their host specificity cannot be accepted with certainty, particularly that of *Trichobius sparsus* and *Joblingia schmidti*. These two species have been found on common cave bats, of which *Chilonycteris rubiginosa rubiginosa*, as has already been stated, sometimes roosts amongst other bats. It is even possible that the bats on which they have been found are not their specific hosts. It seems that the host specificity in these parasites is poorly developed, and the restriction of some of their species to certain bats is governed not so much by innate physiological factors, as by the roosting habit of these bats.

The American Streblidae, as the Nycteriboscinae of the Old World, never seem to occur in large numbers on a single bat; but the species of the genus *Ascodipteron*, which are endoparasites of some bats of the Old World, may occasionally be present in enormous numbers. A single American bat very rarely harbours more than eight or ten Streblids; more often it is parasitized by two or three specimens only. Many bats of those species which can harbour these parasites are very often not infested at all. But nearly all the specimens of the species of *Desmodus*, *Noctilio*, and especially of *Carollia*, *Chilonycteris* and *Phyllostomus*, which have been examined by Mr Jones in Trinidad, carried one or two specimens of these ectoparasitic flies.

When a single specimen is present on a bat it is almost without exception a female, and if there are two then one of them is usually a young female and the other a young male. On more infested bats the females are always much more numerous than the males, and many of them represent the old

specimens.* The presence of many more females than males shows that the latter are short-lived; they probably die soon after the fertilization of the females.

The foregoing description shows that the roosting habit of bats has been a very important factor in the adaptation of many species of Streblidae to the different species of bats. Another factor which could influence this adaptation is the peculiar mode of reproduction of these flies. They all produce the fully developed, immobile larvae (prepupae), one at a time. In the Old World genus *Ascodipteron* the immobile larva is dropped to the ground, where it immediately forms the puparium. The habit of dropping the larva is probably present only in this genus, because its females are endoparasitic, they live embedded in the skin of bats, and are unable to leave their hosts (Muir, 1912; Jobling, 1939*b*, 1940). But in all the other genera of the Streblidae the larva is probably attached by the female to an object not far from the roosting bats, where it forms the puparium. The puparia of *Trichobius coecus* and *T. mixtus* were described by Guimarães (1944), and they have been found attached to a block of the wall.

As regards this breeding habit it appears that the imago emerging in the cave frequented by bats will have a very good chance of finding its host. This can be either the same species or some other bat, which may belong to a different genus or even to a different family of Chiroptera. This kind of infestation is probably more often brought about by an occasional exchange of roosting places between two different species of bats.

An exchange of these parasitic flies can take place not only between the cave bats, but also between cave and forest bats. One case of this exchange occurs between the fruit-eating, forest bat, *Uroderma bilobatum* Peters and the cave bat, *Artibeus jamaicensis jamaicensis* Leach. The first species usually roosts under banana leaves, but in the Canal Zone, Panama, according to Barbour (1932), it passes the day in special shelters, which it builds from the fronds of palm leaves. This species is parasitized by *Paratrichobius dunni*, whereas *Artibeus jamaicensis jamaicensis* is parasitized by the other species of the same genus, *Paratrichobius longicrus*, which is also almost as common on the other cave bat, *Carollia perspicillata perspicillata* L. However, there is one record of *Paratrichobius dunni* from *Artibeus jamaicensis jamaicensis*, and of *Paratrichobius longicrus* from *Uroderma bilobatum*. The occurrence of these two Streblids on the bats which are not their usual hosts is explained by the fact that although *Artibeus jamaicensis jamaicensis* is a cave-dweller, it very often roosts in the forest, where occasionally it may use the same roosting place as *Uroderma bilobatum*. As *Artibeus jamaicensis jamaicensis* is the host of the other six species of Streblidae, it is quite possible that some of them may also be found on *Uroderma bilobatum* and on some other forest bats.

There is also the morphological factor which influences the relationship between these parasites and their host. The great majority of Streblidae have well-developed, functional wings, which they can use in searching for their host. But in the species of the genus *Aspidoptera*, in *Pterellipsis aranea*, and in *Metelasmus pseudopterus* the wings are greatly reduced and are not functional, whereas in *Paradyschiria dubia* they are completely absent. In all these species, except *P. aranea*, the legs are short and strong, and are more adapted for clinging to the host than for crawling. Therefore, the survival of these species must depend more upon the habits of their hosts to frequent the same roosting places than the survival of the winged species. These Streblidae are restricted to one or two species of bats; they rarely occur on those bats which are not their principal hosts. For example, *Aspidoptera megastigma* is restricted to *Noctilio leporinus* L., and *Paradyschiria dubia* to the same species and *Dirias albiventer albiventer* Spix and its one subspecies. These two genera of bats comprise the family Noctilionidae.† Recently, *Paradyschiria dubia* has been recorded from the two species of the Phyllostomidae, *Glossophaga soricina* Pallas and *Anoura geoffroyi* Gray, in Brazil. The occurrence of *Paradyschiria dubia* on these two species of bats is undoubtedly due to the roosting of the Noctilionidae in the same place as the Phyllostomidae. But, curiously enough, there is no record of the Streblidae of the Phyllostomidae from the Noctilionidae, although the bats of the former family are usually much more numerous in the roosting places and are more heavily infested than the bats of the latter family. This is probably due to the smell of the Noctilionidae, which, according to Cott (1926), is very strong, unpleasant, and can be detected more than a hundred yards from their roosting place. It is quite possible that this smell acts as a repellent to the Streblidae of the Phyllostomidae and prevents their migration to the Noctilionidae.

There are many rare species of bats, of which some also have these parasites. According to their infestation with Streblidae they may be divided into three groups. To the first group we may refer all those species which frequent the caves. These are parasitized by the same Streblidae which are common on

* The age of these flies can be judged by the colour of their cuticle, which is more darkly pigmented in the old specimens. In the female it can also be judged by the size of the abdomen, which stretches very considerably during the development of the larva in the uterus, and after the expulsion of the larva it shrinks, but never acquires the same dimensions.

† According to the new classification of mammals the genus *Dirias* is only a subgenus of *Noctilio* (Simpson, 1945).

many other cave bats. Here belong the following species: *Natalus stramineus* Gray and *Pteronotus davyi* Gray, which are parasitized by *Trichobius coecus*, a common species on the cave bat, *Chilonycteris rubiginosa rubiginosa* Wagner; *Phyllonycteris poeyi* Gundlach, which is parasitized by *Trichobius parasiticus*, a very common Streblid on the species of the genus *Desmodus*; *Diphylla centralis* Thomas, of the family Desmodontidae, which, like the other species of this family, is usually parasitized by *Trichobius parasiticus*, and by *Euctenodes mirabilis*; and *Enchistenes harti* Thomas, which is parasitized by *Trichobius dugesii*, a common species on the other genera of the cave-dwelling Phyllostomidae and on one genus of the Desmodontidae.

The second group includes only the three very rare species of bats. Each of them is parasitized by a single species of Streblidae. Thus *Furipterus horrens* Cuvier harbour *Trichobius pallidus*, the species more or less resembling *T. coecus*; *Leptonycteris nivalis* de Saussure is parasitized by *Trichobius sphaeronotus*; *and Amorphochilus schnablii* Peters by *Sythesiostrebla amorphochili*. As regards the host-parasite relationship between these bats and their Streblidae, it can be assumed that they carry on an isolated life from other bats and, therefore, they are neither infested with the Streblidae of these bats nor pass their own parasites to them.

To the third group belongs a very large number of the forest bats, from which there is no record of Streblidae. Their freedom from these parasites is probably due to their complete isolation from those few species of the forest bats which harbour them.

The common occurrence of the Streblidae on the cave bats, their heavy infestation of the latter, and their presence only on a few forest bats rather indicates that they originated in the caves. The ancestors of the Streblidae were non-blood-sucking flies which undoubtedly had some resemblance to the present Acalypterae. Before the development of the blood-sucking habit and a new mode of reproduction, these flies were probably breeding in the bats' dung, which started to accumulate on the floor of the caves, after the adaptation of several species of the arboreal bats to roosting in caves. Therefore it seems that the absence of Streblidae on forest bats indicates that these bats still retained their primitive habit of roosting, whereas the presence of these parasites points to the reversion of these bats to their primitive habit. The parasitized forest bats have probably acquired their Streblidae when they were still cave dwellers.

KEY TO THE GENERA OF THE NEW WORLD STREBLIDAE

1. Head without ctenidium (comb-like row of strong spines) 2
   Head with ctenidium (Fig. 3B, D, F) 12
2. Wings normal, with six longitudinal and three cross veins (Fig. 1A, B) 3
   Wings otherwise or absent 8
3. Fore femora with diagonal row of strong, spine-like setae (Fig. 1B, C) . *Paratrichobius* Costa Lima
   Fore femora without diagonal row of spine-like setae 4
4. Head more or less rounded or piriform, narrower than the anterior margin of the thorax (Fig. 2A) 5
   Head more or less trapezoidal and as broad or nearly as broad as the anterior margin of the thorax (Fig. 2D, E, F) 6
5. Hindlegs not elongated . . . . . *Trichobius* Gervais
   Hindlegs strongly elongated, twice as long as the forelegs (Fig. 2B) . . . . *Speiseria* Kessel
6. Postero-dorsal margin of the head with two rounded, flap-shaped projections overlapping the thorax. Median suture of the prescutum absent (Fig. 2D) . . . . . *Stizostrebla* Jobling
   Head without flap-shaped projections. Median suture of the prescutum present (*m*) 7
7. Anterior margin of the prescutum produced and sinuate in the middle. Humeral caluses small (Fig. 2F) . . . . . *Pseudostrebla* Costa Lima
   Anterior margin of the prescutum with two sharp, triangular teeth in the middle. Humeral caluses very large and projecting under the head (Fig. 2E) . . . . . *Synthesiostrebla* Townsend
8. Wings longer than the abdomen, with three longitudinal and one cross vein. Body strongly compressed (Fig. 1D) . . . . . *Nycterophilia* Ferris
   Wings greatly reduced, shorter than the abdomen, or absent (Fig. 1E, F, G, I and J) 9
9. Thorax much longer than broad, its lateral parts almost flat and vertical. Hindlegs more than twice as long as the forelegs (Fig. 1E, Fig. 2C) . . . . . *Pterellipsis* Coquillett
   Thorax broader than long. Hindlegs not elongated 10
10. Wings present. Femora uniform; tibiae without lightly pigmented ring 11
    Wings absent. Femora of the forelegs much stronger than those of the other legs; tibiae with lightly pigmented ring (Fig. 3A) . . . . . *Paradyschiria* Speiser

KEY TO THE GENERA OF THE NEW WORLD STREBLIDAE (*continued*)

11. Dorsal surface of head divided into distinct sclerotized subregion (Fig. 1H). Legs short and very strong; tibiae as long as or a little longer than the tarsi . . . . . *Aspidoptera* Coquillett
    Subregions of the head not well defined. Legs long; tibiae more than twice as long as the tarsi (Fig. 3G, H) . . . . . *Joblingia* Dybas & Wenzel
12. Postero-ventral margin of the head with ctenidium of 18–19 spines (Fig. 3B) . *Eldunnia* Curran
    Ctenidium composed of numerous spines and extending on to the dorso-lateral parts of the head (Fig. 3D, F) 13
13. Eyes composed of a number of facets. Wings normal 14
    Eyes reduced to a single not quite distinct facet. Wings reduced to small flaps (Fig. 3C) . . *Metelasmus* Coquillett
14. Postero-dorsal margin of frons not overlapping the antennae. Palpi slightly longer than broad, triangular (Fig. 3F) . . . . . *Strebla* Wiedemann
    Postero-dorsal margin of frons overlapping the antennae. Palpi broader than long (Fig. 3D, E) . 15
15. All femora strong; hindlegs less than twice as long as the forelegs . . . *Euctenodes* Waterhouse
    Hindlegs twice as long as the forelegs, their femora long and slender
    Paraeuctenodes Pessôa & Guimarães

Table 2. *Showing the species of bats on which the species of Streblidae have been found in Trinidad, British West Indies*

Families, genera and species of bats	Genera and species of Streblidae														
	Trichobius coecus Edwards	*T. perspicillatus* Pessôa & Galvão	*T. parasiticus* Gervais	*T. dugesii* Townsend	*T. mixtus* Curran	*Paratrichobius longicrus* Ribeiro	*Speiseria ambigua* Kessel	*Pterellipsis aranea* Coquillett	*Nycterophilia coxata* Ferris	*Aspidoptera clovisi* Pessôa & Guimarães	*A. megastigma* Speiser	*Paradyschiria dubia* Rudow	*Euctenodes mirabilis* Waterhouse	*Strebla vespertilionis* Fabricius
NOCTILIONIDAE														
Noctilio leporinus L.	.	.	.	.	.	.	.	.	.	.	+	+	.	.
PHYLLOSTOMIDAE														
Anoura geoffroyi geoffroyi Gray	.	.	.	.	+	.	.	.	.	+	.	.	.	.
Artibeus planirostris trinitatis K. And.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
A. jamaicensis palmarum Allen & Chapman	.	.	.	.	.	+	.	.	.	.	.	.	.	.
Carollia perspicillata perspicillata L.	.	.	.	+	.	.	+	.	.	.	.	.	+	.
Chilonycteris rubiginosa rubiginosa Wagner	+	.	+	+	.	.	+	.	+	.	.	.	+	.
Enchistenes harti Thomas	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Glossophaga soricina Pallas	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Phyllostomus discolor Wagner	.	+	.	.	+	.	.	.	.	.	.	.	+	.
P. hastatus hastatus Pallas	.	.	.	.	+	.	.	.	.	.	.	.	+	.
DESMODONTIDAE														
Desmodus rotundus rotundus Geoffroy	.	.	+	.	.	.	.	.	.	.	.	.	+	+

RECORD OF THE STREBLIDAE FROM TRINIDAD

Only the following species of the Streblidae had been known from Trinidad before: *Trichobius coecus* Edwards, *T. dugesii* Townsend, *Aspidoptera clovisi* Pessôa & Guimarães and *A. megastigma* Speiser. The present record includes nine genera with fourteen species. Of these genera, *Speiseria*, *Pterellipsis*, *Nycterophilia*, *Paradyschiria* and *Strebla* are monotypic.

It is shown in Table 2 that the Streblidae of Trinidad parasitize eleven species of bats which

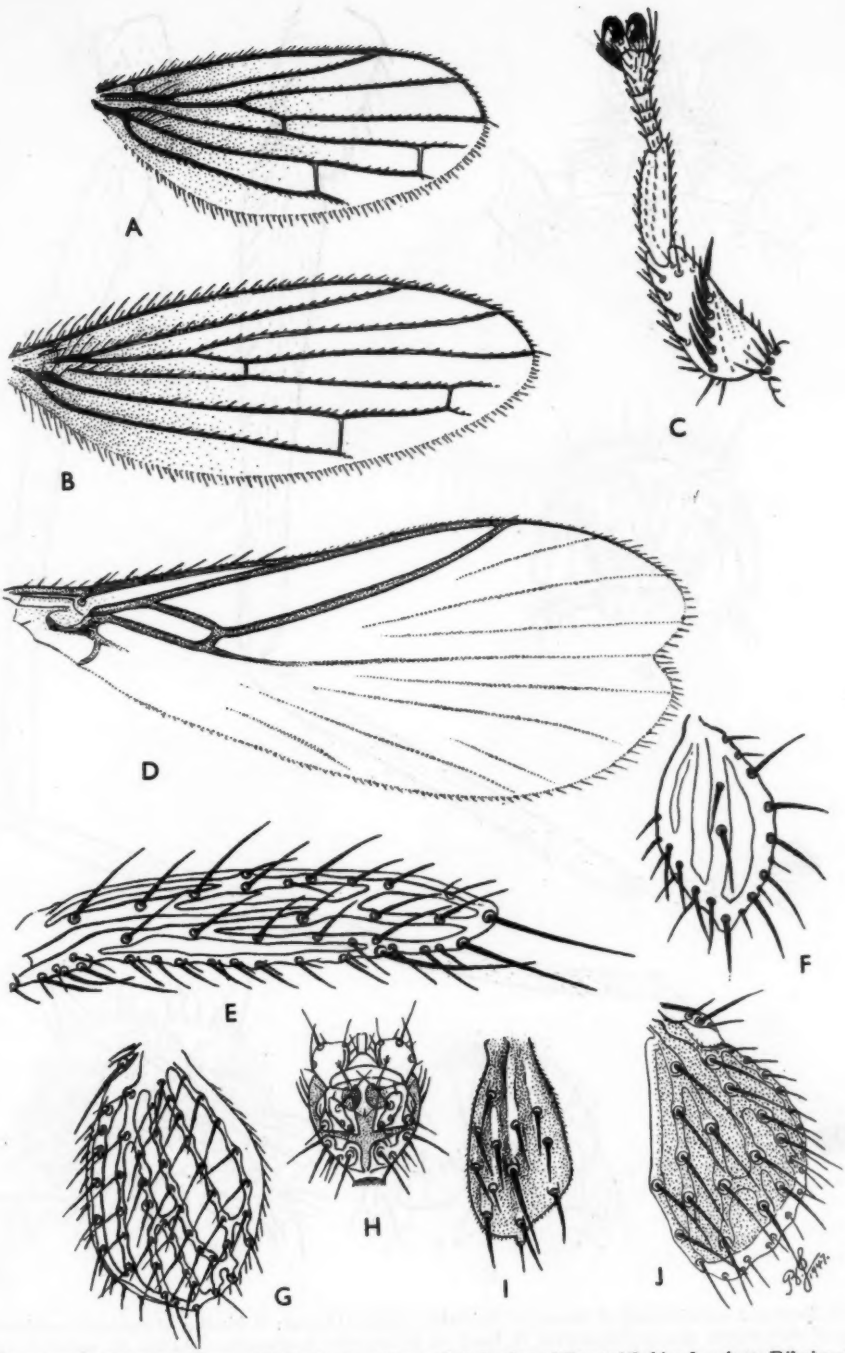


Fig. 1. A, wing of *Trichobius coecus* Edwards; B, wing and C, foreleg of *Paratrichobius longicrus* Ribeiro; D, wing of *Nycterophilia coxata* Ferris; E, same of *Pterellipsis aranea* Coquillett; F, same of *Aspidoptera megastigma* Speiser; G, same of *A. phyllostomatis* Perty; H, head of *A. megastigma*; I, wing of *A. minuta* Costa Lima; J, same of *A. clovisi* Pessôa & Guimarães.

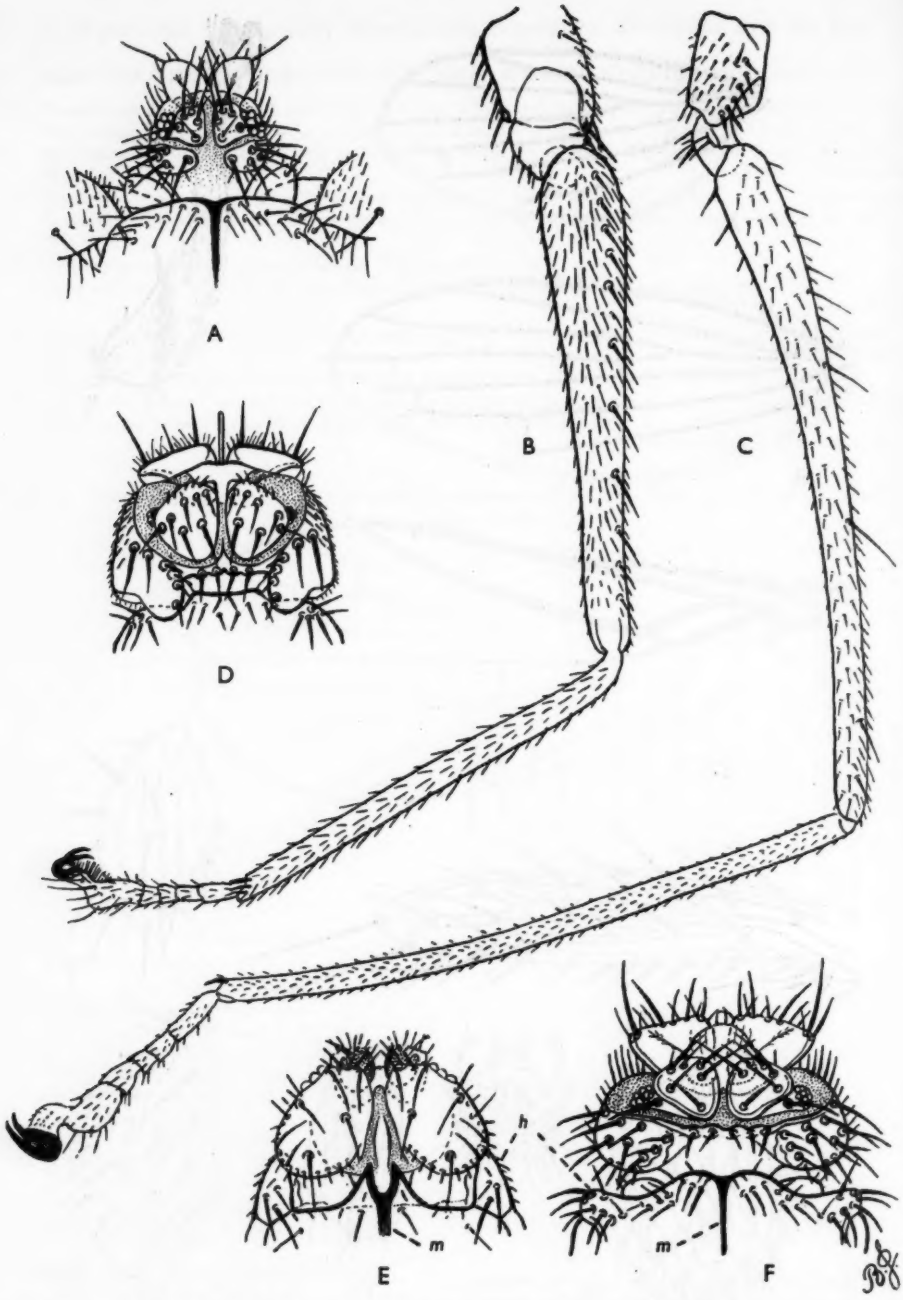


Fig. 2. A, head and anterior part of thorax of *Trichobius mixtus* Curran; B, hindleg of *Speiseria ambigua* Kessel; C, same of *Pterellipsis aranea* Coquillett; D, head of *Stizostrebla longirostris* Jobling; E, same of *Synthesiostrebla amorphochili* Townsend; F, same of *Pseudostrebla riberoi* Costa Lima; *h*, humeral caluses; *m*, median suture of prescutum.

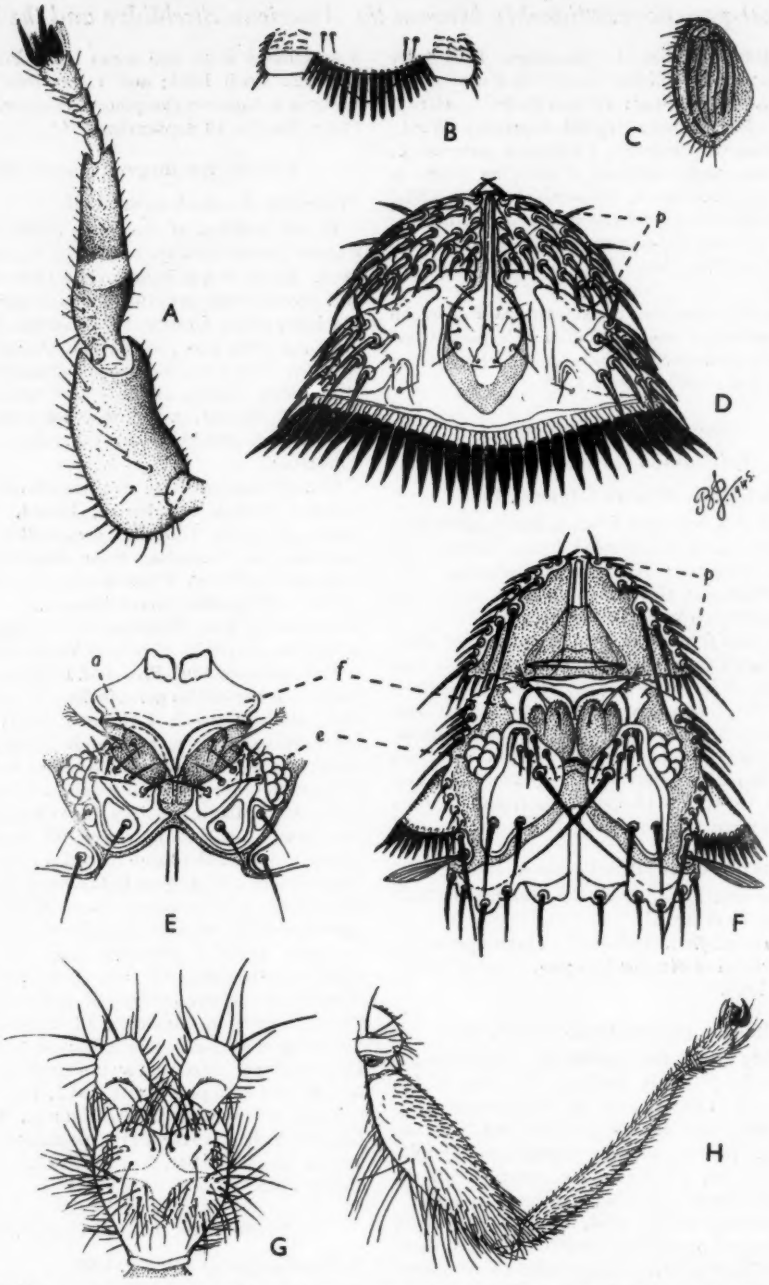


Fig. 3. A, foreleg of *Paradyschiria dubia* Rudow; B, posterior margin of ventral surface of head of *Eldunnia breviceps* Curran (after Curran, 1935); C, wing of *Metelasmus pseudopterus* Coquillett; D, ventral view of head of *Euctenodes mirabilis* Waterhouse; E, frons and part of vertex of same; F, dorsal view of head of *Strebla vespertilionis* Fabricius; G, head and H, foreleg of *Joblingia schmidti* Dybas and Wenzel (after Dybas & Wenzel, 1947); *a*, antennae; *e*, eyes; *f*, frons; *p*, palpi.

belong to three families of Chiroptera. It is quite possible that the Streblidae will also be found on the following bats in Trinidad: *Natalus stramineus* Gray, *Pteronotus davyi* Gray, *Myotis nigricans* Wied., *Sturnia lilium* Geoffroy and *Vampyrus spectrum* L. The first two species harbour *Trichobius coecus* in Jamaica and Dominica, respectively; the third species, *Trichobius pseudotruncatus*, in Dominica; the fourth species, *Aspidoptera clovisi*, and the fifth species, *Trichobius parasiticus*, on the American mainland.

As in the other parts of the Neotropical region the greatest number of the Streblidae of Trinidad has been found on the species of bats which belong to the family Phyllostomidae (Table 1).

Subfamily TRICHOBIINAE

Trichobius Gervais, 1844

Trichobius coecus Edwards, 1918

The type was collected from a bat in Trinidad. The species has been recorded from *Chilonycteris rubiginosa rubiginosa* Wagner, in Panama; from *Natalus stramineus* Gray, in Jamaica; and from *Pteronotus davyi* Gray, in Dominica.

Present record from Trinidad. One specimen from *Chilonycteris rubiginosa rubiginosa* Wagner, Cave, Caura Valley, 3 December 1944; 23 specimens from the same host, Casti Cave, Diego Martin, 19 September 1944; 7 specimens from the same host, Oropuche Cave, Cumaca, 30 August 1944; 16 specimens from the same host, Cave, Heights of Guanapo, 18 October 1944; and 11 specimens from the same host, Aripo Cave 3, 4 November 1944.

Trichobius perspicillatus Pessôa & Galvão, 1937

The type was taken from *Carollia perspicillata perspicillata* L. in Brazil.

Present record from Trinidad. Three specimens from *Phyllostomus discolor* Wagner, Port-of-Spain, 5 October 1944.

Trichobius parasiticus Gervais, 1844

This widely distributed species has been recorded from Argentina, Brazil, Guiana, Panama, Cuba, Paraguay and Yucatan. It is very common on *Desmodus rotundus rotundus* Geoffroy and *D. rotundus murinus* Wagner, and also parasitizes *Diphylla centralis* Thomas, *Phyllonicteris poeyi* Gundlach, *Vampirus spectrum* L. and *Myotis simus* Thomas.

Present record from Trinidad. One specimen from *Desmodus rotundus rotundus* Geoffroy, El Soconosco Cave, Diego Martin, 19 September 1944; 5 specimens from the same host, Gold-mine shaft, Arima, Blanchisseuse Road, 18 October 1944; 1 specimen from the same host, Valsayn Drain, San Juan, 19 September 1944; 4 specimens from the same host, Silk-cotton-tree, Diego Martin, 21 August 1944; 5 specimens from the same host, Pointe Gourde Cave, 25 April 1945; and 1 specimen from *Chilonycteris rubiginosa rubiginosa* Wagner, Casti Cave, Diego Martin, 19 September 1944.

Trichobius dugesii Townsend, 1891

Trichobius blandus Curran, 1935

In my revision of the genus (1938) I placed *T. mixtus* Curran into synonymy of *T. dugesii* Townsend. My error was indicated by Guimarães (1941), and also by Bequaert (1942), who examined the type specimen in the University of Kansas, and found it identical with the form which Curran named *T. blandus*. Therefore, the forms redescribed by me as *T. blandus* Curran should have the name of *T. dugesii* Townsend, and those which were redescribed as *T. dugesii* should be called *T. mixtus* Curran (see *T. mixtus*).

The species has been recorded from *Glossophaga soricina* Pallas, in Mexico, Brazil, Guiana and Panama; from *Carollia perspicillata azteca* de Saussure, in Panama; from *Desmodus rotundus rotundus* Geoffroy, *Phyllostomus hastatus hastatus* Pallas and *Carollia perspicillata* L., in Brazil; from *Enchisthenes harti* Thomas, in Trinidad; and from an unknown species of bat, in Venezuela.

Present record from Trinidad. Five specimens from *Carollia perspicillata perspicillata* L., under Bridge, Blanchisseuse Road, 1 October 1944; 1 specimen from the same host, Springhill Estate, 1 October 1944; 4 specimens from the same host, Maraval Cave, 5 October 1944; 16 specimens from the same host, Diego Martin, 28 July 1944; 6 specimens from the same host, St Augustine, 23 August 1944; 3 specimens from the same host, La Fountaine Cave, Diego Martin, 21 August 1944; 8 specimens from the same host, El Soconosco Cave, 22 March 1945; 1 specimen from the same host, Pointe Gourde Cave 2, 25 April 1945; 1 specimen from the same host, Griffith Settlement, 17 July 1944; and 1 specimen from *Chilonycteris rubiginosa rubiginosa* Wagner, Cave, Heights of Guanapo, 18 October 1944. The following specimens were collected by Mr P. C. J. Bruent: 1 specimen from an unknown species of bat, No. XX0845, Lopinot, VIII, 1945; 1 specimen from *Carollia perspicillata perspicillata* L., 'Devils hole', Lopinot Valley, 1945; and 4 specimens from an unknown species of bat, No. 010745AA, Caura Cave, 1 July 1945.

Trichobius mixtus Curran, 1935

Trichobius dugesii Jobling, 1938

In my revision of the genus (1938) it was redescribed under the name of *T. dugesii* (see *T. dugesii*). The species has been found on *Artibeus jamaicensis jamaicensis* Leach, in Porto Rico, and on its subspecies, in the Bahamas, Cuba, and Yucatan

on *Carollia perspicillata azteca* de Saussure and *Phyllostomus hastatus panamensis* Allen, in Panama; on *Carollia perspicillata perspicillata* L. and *Phyllostomus hastatus hastatus* Pallas, in Brazil; and also on the last-mentioned bat, in Venezuela. Its record from *Chilonycteris rubiginosa rubiginosa* Wagner in Panama is rather doubtful. It had been made before the separation of this species from *T. dugesii*.

Present record from Trinidad. Twenty specimens from *Phyllostomus discolor* Wagner, Port-of-Spain, 5 October 1944; 13 specimens from *Anoura geoffroyi geoffroyi* Gray, Aripo Cave, 2 July 1944; 1 specimen from the same host and locality, 11 June 1945; and 8 specimens from *Phyllostomus hastatus hastatus* Pallas, Jumbie Cave, Caura Valley, 18 July 1944.

Paratrichobius Costa Lima, 1921

Paratrichobius longicrus Ribeiro, 1907

The type was collected from *Artibeus jamaicensis jamaicensis* Leach, in Brazil. It has also been recorded from a bat, in Mexico; from *Vampyrops lineatus* Geoffroy, in Paraguay; from the same host and *Carollia perspicillata perspicillata* L., in Brazil; from *Artibeus jamaicensis palmarum* Allen and Chapman, in Venezuela; and from *Uroderma bilobatum* Peters, in Panama.

Present record from Trinidad. Two specimens from *Artibeus jamaicensis palmarum* Allen & Chapman, St Augustine, 26 November 1944.

Speiseria Kessel, 1925

Speiseria ambigua Kessel, 1925

In my paper (1939*a*) it was incorrectly identified as *Synthesiostrebla amorphochili* Townsend. This mistake has been recently corrected in my other paper (1947).

The type was taken from a bat in Pernambuco. The species has also been recorded from *Carollia perspicillata perspicillata* L., in Panama; from *Tonatia bideus* Spix, in British Guiana; from *Glossophaga soricina* Pallas and *Carollia perspicillata perspicillata* L., in Brazil; and from a bat in Venezuela.

Present record from Trinidad. One specimen from *Carollia perspicillata perspicillata* L., under bridge, Blanchisseuse Road, 1 October 1944; 1 specimen from the same host, Springhill Estate, 1 October 1944; 1 specimen from the same host, Maraval Cave, 5 October 1944; 2 specimens from the same host, Diego Martin, 28 July 1944; 4 specimens from the same host, La Fountaine Cave, Diego Martin, 21 August 1944; and 1 specimen from *Chilonycteris rubiginosa rubiginosa* Wagner, Cave, Heights of Guanapo, 18 October 1944. Mr T. S. Jones informed me that Dr Bequaert has seen one specimen from an unknown species of bat collected by E. B. Williamson, at Arima.

Pterellipsis Coquillett, 1899

Pterellipsis aranea Coquillett, 1899

Megistopoda desiderata Speiser, 1900
Pterellipsis proxima Seguy, 1927, n.syn.

The species has been recorded from an unknown species of bat in Cuba, Porto Rico, West Indies and Venezuela; from *Artibeus jamaicensis jamaicensis* Leach, in Canal Zone and Panama; from *A. planirostris polax* Peters, in British Guiana; and from *Phyllostomus* sp., in Brazil. Mr T. S. Jones collected 1 specimen from *Artibeus planirostris trinitatis* K. And., in Charlotteville, Tobago, 27 February 1945.

Present record from Trinidad. One specimen from a bat, St Augustine, 25 April 1943; 8 specimens from *A. planirostris trinitatis* K. And., St Augustine, on 3 February 1945, 23 June 1945 and on 8 May 1945.

Nycterophilia Ferris, 1916

Nycterophilia coxata Ferris, 1916

The type was collected from *Macrotus californicus* Baird, California. It has also been recorded from *Brachyphilla cavernarum* Gray, in British West Indies; from *Chilonycteris rubiginosa rubiginosa* Wagner, in Panama; from *Artibeus jamaicensis jamaicensis* Leach, in Inagua Id., Bahamas; and from *Chilonycteris personata* Wagner, in Brazil.

Present record from Trinidad. Fifteen specimens from *Chilonycteris rubiginosa rubiginosa* Wagner, Casti Cave, Diego Martin, 19 September 1944.

Aspidoptera Coquillett, 1899

Aspidoptera clovisi Pesôa & Guimarães, 1936

It has been recorded from *Anoura geoffroyi geoffroyi* Gray, in Brazil and Trinidad; from *A. geoffroyi apolinari* Allen (*A. geoffroyi apolinari* is a synonym of *A. geoffroyi peruana* Tschudi), in Colombia; and from *Sturnia lilium* Geoffroy, in Paraguay.

Present record from Trinidad. From *Anoura geoffroyi geoffroyi* Gray, Aripo Cave, 4 specimens on 29 November 1944, 23 specimens on 2 July 1944, and 24 specimens on 11 June 1945.

Aspidoptera megastigma Speiser, 1900

This species has been recorded from *Noctilio leporinus* L., in Trinidad, Cuba and Venezuela. The name of the host of the Venezuelian specimens was given by Rudow (1871) as *N. dorsatus* Wied., which is a synonym of *N. leporinus* L.

Present record from Trinidad. Thirty-nine specimens from *N. leporinus* L., Pointe Gourde Cave, 25 April 1945.

Paradyschiria Speiser, 1900

Paradyschiria dubia Rudow, 1871

Paradyschiria fusca Speiser, 1900
Paradyschiria lineata Kessel, 1925, n.syn.
Paradyschiria parvula Falcoz, 1931, n.syn.

After an examination of many specimens from different parts of America, and also the paratypes of *P. lineata* Kessel, I came to the conclusion that there is only one species of *Paradyschiria*. Speiser (1900) overlooked the paper of Rudow, and in his subsequent paper (1902) regarded his *P. fusca* as a synonym of *P. dubia* Rudow. I have no doubt that *P. parvula* Falcoz is a very small male specimen of *P. dubia*. A very good illustrated redescription of this species is given by Guimarães (1941).

The type was collected from *Noctilio dorsatus* Wied. (*N. dorsatus* is a synonym of *N. leporinus*), in Venezuela. It has been recorded from the same host, in Colombia and Brazil; from *Glossophaga soricina* Pallas, *Anoura geoffroyi geoffroyi* Gray, and *Dirias albiventer albiventer* Spix, in Brazil; and from *D. albiventer minor* Osgood, in Panama.

Present record from Trinidad. Six specimens from *Noctilio leporinus* L., Pointe Gourde Cave, 25 April 1945.

Subfamily STREBLINAE

Strebla Wiedemann, 1824

Strebla vespertilionis Fabricius, 1805

According to Speiser (1900) this species occurs on the following species of bats: *Vampirops lineatus* Geoffroy, and *Lonchoglossa caudifera* Geoffroy, and he mentions the following localities: Colombia, South America, Jamaica and Mexico. It has also been recorded from *Anoura geoffroyi geoffroyi* Gray, in Brazil; *A. geoffroyi apolinari* Allen, (*A. geoffroyi apolinari* Allen is a synonym of *A. geoffroyi peruana* Tschudi), in Bogota, Colombia; and from an unknown species of bat, in Venezuela; there is one record of this species from *Desmodus rotundus rotundus* Geoffroy, in Trinidad. According to Mr T. S. Jones the specimens were collected by D. O'Connor in 1939, and identified by Dr J. Bequaert.

Euctenodes Waterhouse, 1879

Euctenodes mirabilis Waterhouse, 1879

The type was found on a bat in Colombia. It has also been recorded from *Carollia perspicillata perspicillata* L., in Pernambuco; from *C. perspicillata azteca* de Saussure and *Phyllostomus hastatus panamensis* Allen, in Panama; from *P. hastatus hastatus* Pallas, in Peru; from *Diphylla centralis* Thomas, in Yucatan; from *Tonatia bidens* Spix, in British Guiana; from *Desmodus* sp., in Peru and Brazil; from an unknown species of bat, in Venezuela; and from an opossum, *Glironia venusta*, in Bolivia. I think that the host in the last-mentioned record was purely accidental.

Present record from Trinidad. Eleven specimens from *Desmodus rotundus rotundus* Geoffroy, El Soconosco Cave, Diego Martin, 19 September 1944; 5 specimens from the same host, Gold-mine shaft, Arima, Blanchisseuse Road, 18 October 1944; 2 specimens from the same host, Valsayn Drain, San Juan, 19 September 1944; 3 specimens from the same host, Silk-cotton-tree, Diego Martin, 21 December 1944; 3 specimens from the same host, Pointe Gourde Cave, 25 April 1945; 3 specimens from *Phyllostomus hastatus hastatus* Pallas, Cave, Heights of Guanapo, 18 October 1944; 21 specimens from *P. discolor* Wagner, Port-of-Spain, 5 October 1944; 1 specimen from *Carollia perspicillata perspicillata* L., Springhill Estate, 1 October 1944; 4 specimens from the same host, La Fountaine Cave, Diego Martin, 21 August 1944; 1 specimen from the same host, Diego Martin, 28 July 1944; 3 specimens from the same host, El Soconosco Cave, 22 March 1945; 1 specimen from *Chilonycteris rubiginosa rubiginosa* Wagner, Cave, Heights of Guanapo, 18 October 1944; 1 specimen from *Glossophaga soricina* Pallas, Pointe Gourde Cave, 25 April 1943; and 1 specimen from an unknown species of bat, Caura Cave, 1 July 1945. According to Mr T. S. Jones, Dr Bequaert has seen one specimen from an unknown species of bat, collected by E. B. Williamson, at Arima.

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STUDIES ON THE VIRUS OF TURNIP YELLOW MOSAIC

By ROY MARKHAM and KENNETH M. SMITH, *Plant Virus Research Unit, Molteno Institute, University of Cambridge*

(With Plates IV–VII and 4 Figures in the Text)

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INTRODUCTORY

There are several viruses, in addition to the virus of cucumber mosaic, which attack cruciferous crops in the British Isles (Smith, 1937). These are, however, if not actually related, of the aphis-transmitted type, of low concentration and stability and are unsuited for biochemical studies. The virus which is dealt with in this paper is, so far as we know, a new one and has not been described before, except in two preliminary communications (Markham & Smith, 1946; Smith & Markham, 1946). It is, as will be seen, of a completely different type from the aphis-transmitted crucifer viruses and is very suitable for more fundamental studies.

SYMPTOMATOLOGY

The name which we suggest for the disease is *turnip yellow mosaic* because of the outstanding yellow mottling which is the most characteristic symptom. The first signs of infection develop from 10 to 14 days after inoculation and take the form of a pronounced yellow clearing of the veins on the youngest leaves (Pl. IV, fig. 4). This is followed by the appearance of small yellow patches on the older leaves and these coalesce and give rise to large yellow areas. In certain host plants, notably Chinese cabbage (*Brassica chinensis*), the mosaic is so pronounced as to resemble a variegation consisting of intense yellow or white patches intermingled with dark green areas (Pl. V, fig. 1).

Local chlorotic lesions do develop on occasion, but their appearance is uncertain and they cannot be depended upon for accurate quantitative work. Their development is most regular on Chinese cabbage under winter conditions and, if the plants are deficient in nitrogen, they appear as dark purple circles. Attempts to find a host plant which develops local lesions with more regularity have so far failed. On the turnip plant the yellow mosaic is very similar to that on Chinese cabbage, but is slightly less intense. On swedes, cabbages and cauliflowers the mottling is much brighter than that due to the aphis-transmitted viruses, but is still less pronounced than on Chinese cabbage or turnips.

The flowers of infected Chinese cabbage plants show a white 'break', the yellow flowers having white stripes or flecks, whilst on occasion they may be entirely white. Infected plants are small and stunted as compared with the normal.

HOST RANGE AND DISTRIBUTION

The virus appears to be confined to the Cruciferæ and all attempts to infect plants of other families by inoculation or by means of dodder (*Cuscuta* spp.) have so far failed.

The virus has been found occurring naturally on turnips, swedes, and broccoli. In addition to these three it has been transmitted experimentally to radish, *Brassica carinata*, *B. juncea*, *B. arvensis* and *B. nigra*, Chinese cabbage of many different varieties, kohlrabi, cabbage, shepherd's purse (*Capsella bursa-pastoris*), and watercress (*Nasturtium* spp.).

The virus has been recorded from the Bristol area and from the Edinburgh district, where it seems to be fairly common and widespread. It also occurs commonly in Portugal and virus material kindly sent us from there by Mrs d'Oliveira proved to be serologically identical.

THERMAL INACTIVATION POINT

In a number of tests we found that the virus in the sap became inactivated after being heated for 10 min. at temperatures between 70 and 75° C.

DILUTION END-POINT

Positive infections were obtained fairly regularly at dilutions of 10^{-5} and not infrequently at dilutions of 10^{-6}. There seems little doubt that the virus content of the sap is variable and depends upon cultural conditions of the plants.

TRANSMISSION

The virus is very infectious and is easily transmitted by sap-inoculation. Owing to the occurrence of a few unexplained infections among seedlings of Chinese cabbage, it has been suggested that a small percentage of seed transmission might occur. We have, however, been unable to confirm this and, in view of the new information on insect transmission, there may be an alternative explanation for infections among seedlings.

INSECT RELATIONSHIPS OF THE VIRUS

As is usual when exploring the insect relationships of a new plant virus, the first insect to be tested as a potential vector was the aphis, *Myzus persicae* Sulz. Contrary to expectation, the large number of experiments made with this insect were negative, as were those with the mealy cabbage aphis, *Brevicoryne brassicae*. Since capsid bugs had been fairly common in the turnip plots, three species of capsids, *Lygus pabulinus*, *L. pratensis* and *Calocoris norvegicus*, were tested, but again with negative results. It appeared, therefore, since no other hemipterous insects were observed among the turnips in sufficient quantities, that sucking insects played no part in the spread of the virus.

The next step was to find out if the virus would actually spread under field conditions. To this end a rectangular plot of ground was sown with turnip seed and, when the seedlings were sufficiently advanced, a few infected seedlings were planted at random in the plot. It was soon evident that the virus was spreading and the rate of spread is dealt with in a later section. Examination of the insect fauna of the plot showed that the only insects present in sufficient numbers to account for the rapidity of spread were various species of turnip flea-beetles (*Phyllotreta* spp.).

Two species, *Phyllotreta undulata* and *P. cruciferae*, were selected for the test and the following experimental methods were employed. The beetles were colonized, about a dozen at a time, on small infected plants of turnips or Chinese cabbage and confined under a glass lamp chimney with a muslin or cellophane top, muslin being more suitable since the beetles were inclined to eat their way out through the cellophane. After feeding on the source of virus for the requisite time, the insects were transferred by means of an insect catcher to the experimental plants. It was soon evident that both species of beetle were efficient vectors of the virus, primary symptoms of the disease developing 10–14 days after the feeding of infective insects.

Further experiments showed that the transmission was not of the non-persistent type since the beetles were able to retain the virus for 72 hr. and to infect three plants in succession at 24 hr. intervals.

Single beetles were sufficient to infect a plant and preliminary experiments suggest that starving before infection feeding does not materially affect transmission. Beetles starved for 48 hr. after infection feed were still infective though, as would be expected, the number of positive infections showed a decrease as compared with unstarved beetles. Beetles could pick up the virus in 24 hr. and retain it for 3 days when starved. Details of the experiments are given in Tables 1–3.

Tests of other beetle species as vectors

Several other species of flea-beetles, including *Phyllotreta nemorum*, *P. atra* and an unidentified blue species from watercress, were found to transmit the virus. In addition, the mustard beetle (*Phaedon cochleariae*) and its larva were also vectors and a detailed account of some experiments on the transmission of the virus by the larval form of *P. cochleariae* are given in a later paragraph.

Transmission experiments with other types of insects

Since it was clear that more than one species and type of beetle were capable of transmitting the virus, experiments were carried out to test the transmitting power of biting insects not belonging to the Coleoptera.

Orthoptera

Locustidae. Single individuals of the green 'long-horn' grasshopper (*Leptophyes punctatissima* Bosc.) were transferred, after feeding for 48 hr. on an

infected Chinese cabbage, to healthy plants of Chinese cabbage and turnips confined under glass lamp chimneys. In the first experiment, four plants out of twenty colonized on 7 July developed the disease on 31 July. In the second experiment two insects after feeding for 48 hr. on the source of virus were colonized singly for 24 hr. on Chinese cabbage plants and then transferred to a second pair of plants for another 24 hr. In this experiment only the first pair of plants became infected.

Acridiidae. Tests were then made with the common brown 'short-horn' grasshopper of the field (*Stauroderus bicolor* Charp.), but in this case serial transfers were made. About a dozen grasshoppers were colonized on an infected Chinese cabbage plant in a large inverted bell-jar covered with muslin. After feeding for 48 hr. the insects were colonized singly on Chinese cabbage, eight insects and eight plants being used, and three transfers to healthy plants were made at 24 hr. intervals. Out of the twenty-four plants used, twelve became infected, five in the first series of transfers, four in the second and three in the third. These results show that not only is this grasshopper able to transmit the virus, but it can retain it for 3 days and still infect a healthy plant without recourse to a fresh source of virus.

Forficulidae. Similar tests were carried out with the common earwig (*Forficula auricularia* Linn.). After feeding for 48 hr. on an infected Chinese cabbage, seven earwigs were colonized singly on healthy Chinese cabbage seedlings. The insects were placed

Table 1. *Infection tests with various species of biting insects*

Transmitting insect	Exp. no.	Time on source of virus (days)	Time on healthy plant (days)	Positive infections
Phyllotreta undulata	1	6	1	9/12
	2	7	2	8/12
P. cruciferae	1	2	1	4/10
	2	3	1	2/10
P. nemorum	1	3	1	6/12
	2	7	1	2/3
Phaedon cochleariae, adult	1	10	2	2/7
	2	7	2	3/10
P. cochleariae, larva	1	4	2	8/12
	2	5	2	7/7
	3	4	2	7/12
Leptophyes punctatissima Bosc.	1	2	4	4/20
Stauroderus bicolor Charp.	1	2	8	5/8
Forficula auricularia	1	2	2	4/7

Table 2. *Transmission tests with larvae of* Phaedon cochleariae

	Daily transfers			
Larva	1	2	3	4
1	11	–	9	12
2	9	14	10	Pupated
3	11	8	12	–
4	11	–	7	10
5	9	8	–	–
6	–	–	9	–
7	9	14	–	–
8	11	–	–	12
9	11	12	–	–
10	11	–	7	10
11	–	8	11	–
12	–	8	12	11

The numbers in the daily transfer columns indicate the incubation period of the disease in the plant in days. A minus sign indicates no infection.

Table 3. *Transmission tests with larvae of* Phaedon cochleariae

Short infection feed

	Duration of infection feed	Daily transfers			
Larva	(min.)	1	2	3	4
1	1	–	+	+	–
2	1	–	–	+	–
3	1	–	+	–	–
4	3	–	+	–	–
5	3	–	+	–	–
6	3	–	–	–	–
7	5	–	–	–	–
8	5	–	+	–	–
9	5	+	+	–	–
10	10	–	+	–	–
11	10	+	+	–	–
12	10	–	+	+	–

A plus sign indicates a positive infection, a minus no infection.

on the healthy plants on 26 June and on 7 July four out of the seven plants had developed the disease. On 28 June a second experiment was performed using five earwigs which had fed for 24 hr. on the source of virus. On 12 July three out of the five plants colonized with single earwigs, were infected. Serial transfers with this insect have not yet been carried out.

Lepidoptera.

A number of transmission experiments have been carried out with the larvae of the white butterflies, *Pieris brassicae* and *P. rapae*, in which the caterpillars were tested singly and collectively, but without result. Similar tests using a small looper caterpillar, species undetermined, which was found feeding on turnip plants, were also negative.

Transmission experiments with the larva of Phaedon cochleariae

Preliminary experiments proved that this larva is a very efficient vector of the turnip yellow mosaic virus as the following examples show. On 15 May, twelve larvae which had fed for several days on an infected plant of Chinese cabbage were transferred singly to young plants of the same species. On 27 May two plants were infected and by 4 June eight out of the twelve had developed the disease.

In a similar experiment carried out on 27 May, but using two larvae per plant, seven plants out of seven became infected by 1 June. In a third experiment, using single larvae and turnip seedlings instead of Chinese cabbage, seven plants out of twelve were infected.

The next experiments were designed to find out how long the larva could retain the virus without again having access to a source of infection and whether length of feeding time could be correlated with duration of infective power. This involved a number of serial transfers; the experiments, which are set out in Table 2, show the following facts. The larvae easily retained the virus for 4 days and infected the fourth plant in succession; longer periods have not yet been tested. There is a suggestion, it cannot be said to be more than that at present, that length of feeding time does govern the number of plants subsequently infected. For example, in one experiment, larvae, colonized for 48 hr. on the source of virus, carried infection to the fourth successive plant on two occasions out of twelve and infected thirteen plants out of a possible forty-eight. Whereas in the experiment in which the larvae had been bred upon the infected plant, they carried infection to the fourth plant on five occasions out of twelve and infected twenty-nine plants out of a possible forty-eight. On the other hand, some further experiments show that a very brief period of feeding on an infected plant is sufficient to render a larva infective. In the first experiment in this series the larvae were allowed to feed for 1, 3, 5 and 10 min. respectively on the source of virus. They were then transferred four times at 24 hr. intervals to healthy seedlings. In the 1 min. feed series, one larva infected the second and third seedlings, one the third seedling only and one the second only. In the next series of experiments the procedure was varied in that the larvae were allowed to feed for 1 min. only on the infected plant and then transferred eight times at 3 min. intervals to healthy seedlings, remaining overnight on the last plant in the series. There is one point of interest which arises here and that is the very small number of infections which arise in the series of plants in the first 24 hr. transfer, following the 1 min. feeding on the source of virus. In the series of transfers where the feeding time was only 3 min. on each healthy plant, following the 1 min. feed on the virus source, infection developed only in the plant on which the insect remained overnight; though this experiment is admittedly only a preliminary one. In other words, the virus seems to behave like a persistent virus with a short delay in the development of infective power. This question is dealt with further, in the discussion on the insect relationships of the virus.

An attempt was next made to find out whether the virus was retained by the insect during the pupation period and whether the newly emerged adult was infective to healthy plants. A large number of *Phaedon* larvae were bred on Chinese cabbage plants infected with the yellow mosaic and as they became mature were picked off and placed in a closed container, with soil in which to pupate. As each beetle emerged it was confined on a Chinese cabbage seedling under a glass lamp chimney. In all, sixty beetles emerged and were colonized singly on test plants. All the beetles fed, but all the plants remained healthy. This experiment suggests that the virus is not retained through the pupal stage.

Artificial feeding of insects with virus solution

These experiments were carried out largely with flea beetles and two methods were tried. In the first, drops of concentrated virus solution with the addition of a little sucrose were placed directly on the mouthparts of the beetles; this method was unsuccessful and no infection resulted. In the second method, the beetles were given a preliminary starving of 24–48 hr., after which drops of virus solution and sucrose were placed on the sides of a glass vessel containing the beetles. This method was more successful and the beetles fed readily on the drops of virus. In two such experiments the beetles infected three plants out of twelve when colonized singly and five out of twenty-five when colonized two to a plant. The fact that local chlorotic lesions developed round the bite holes on the leaves in one or two cases

suggests that the virus was genuinely transmitted and that infections were not due merely to mechanical contamination.

Similar feeding experiments were carried out with the grasshoppers (*Stauroderus bicolor*), but although the insects fed greedily on the virus solution, they have not, so far, transmitted infection in this way.

DISCUSSION ON INSECT RELATIONSHIPS OF THE VIRUS

The relationship of this virus with its insect vectors is interesting from several points of view. It is the first case recorded of the insect transmission of a crystallizable plant virus; so far as we are aware, not one of the other plant viruses which have been crystallized have an insect vector. It is also the first record, in Europe at all events, of a plant virus being transmitted by an insect with biting mouthparts. There are one or two statements in American literature of the transmission of viruses by biting insects. Doolittle (1920) records that the virus of cucumber mosaic is spread by the 12-spotted cucumber beetle and Goss (1931) states that potato spindle tuber is carried by grasshoppers. In a recent paper Larson & Walker (1939) say that a virus affecting crucifers in Wisconsin, which appears to be somewhat similar to the cabbage black ringspot virus in this country (Smith, 1935), is transmitted by the larvae of *Pieris rapae*, the small white butterfly. We have been unable to confirm this so far as our cabbage black ringspot virus is concerned and it appears to be carried only by one or more species of aphides.

In considering the mode of transmission of the turnip yellow mosaic virus the most likely assumption one would make is that the virus is carried mechanically on the jaws of the various kinds of insects which transmit it. This assumption, however, is not borne out by the experimental results, at least so far as the *Phaedon* larva is concerned.

The experiments suggest that the larvae do not become infective until a period of about 24 hr. has elapsed since the infection feed. Now in the case of persistent viruses transmitted by sap-sucking insects it has been suggested that a similar delay in the development of infective power in the insect is due to the time taken by the virus to pass through the wall of the alimentary canal into the blood and so to the salivary glands. In sucking insects there is little doubt but that the saliva is the vehicle for virus transmission (Carter, 1928; Smith, 1941), but in biting insects like the flea-beetle the situation is different since salivary glands appear to be lacking. Indeed it is significant that in all the insects concerned with the transmission of turnip yellow mosaic virus, with the possible exception of the green grasshopper (*Leptophyes punctatissima* Bosc.), the salivary glands are absent or rudimentary (Bordas, 1898). When feeding, beetles are said to regurgitate part of the contents of the foregut to aid digestion of the leaf tissue and it seems likely that it is during this regurgitation process that infection takes place. It is from this point of view that the lack of salivary glands becomes significant since it is probable that insects without these glands do regurgitate during feeding. This theory is further supported by the fact that lepidopterous larvae which do possess salivary glands are apparently unable to transmit the virus.

A preliminary investigation has been made of the alimentary canals of the transmitting insects, particularly as regards the position of the valves in relation to regurgitation. In aphides, as already known, there is a sub-oesophageal valve which presumably prevents any backward movement of food materials from the gut (Pl. IV, fig. 1). In flea-beetles and in the larvae of *Phaedon cochleariae* the valve is situated at the lower end of the foregut which therefore does not prevent regurgitation. It is a difficult matter to obtain good sections of adult flea-beetles owing to the extreme hardness of the chitin, but Pl. IV, fig. 2 illustrates fairly adequately the valve and its position in the gut of an adult flea-beetle.

Investigations of the alimentary canal of the larva of *P. cochleariae* failed to reveal any salivary glands, but they did suggest that regurgitation of infective material is easy which may be the reason why this larva is such an efficient vector of the virus. Larvae were fixed and sectioned immediately after feeding and in Pl. IV, fig. 3, may be seen in the foregut of a larva the ingested leaf material which appears to lie very close to the mouth.

If such a virus as that of turnip yellow mosaic can be so easily transmitted by a variety of biting insects, it is difficult to understand why the virus of tobacco mosaic and potato virus *X* are not similarly transmitted, since there are no apparent differences in the plant tissue relationships of the viruses and all are in high concentration in the plant and extremely infectious. Experiments to try to induce the potato flea beetle (*Psylliodes affinis*) to transmit potato virus *X* are now in progress.

SPREAD OF THE VIRUS IN THE FIELD

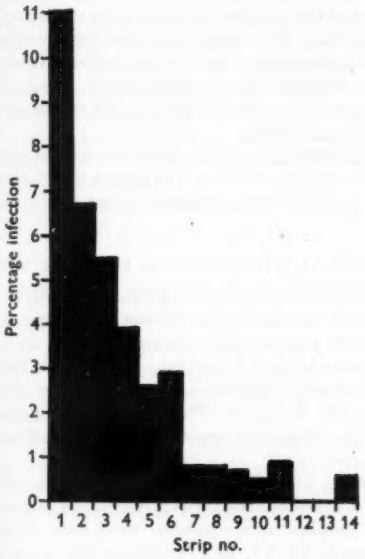
During experiments on the spread of the virus in nature a small plot of turnips was planted outdoors early in 1946 and when the plants were large enough a number were infected artificially. The virus soon spread among the rest and a larger scale experiment was planned.

Adjacent to the first plot a large rectangular plot of turnips was sown, 28 ft. wide and 91 ft. long, the rows being some 17 in. apart and at right angles to the long side of the plot.

Infection was soon observed in the turnip plants in the end nearest the first plot, and it spread

throughout the large plot. Counts of infections made in August showed that 164 plants out of a total of 6840 examined were showing visible symptoms and had therefore been infected at least 3 weeks previously.

The distribution of the infected plants is shown as a histogram in Text-fig. 1. The distribution obtained suggests that the infection is spread by a vector which moves from plant to plant over short distances, but longer than those distances travelled by wingless aphides and much shorter than those travelled by winged aphides, and consistent with a vector having the short active flight of a flea-beetle.



Text-fig. 1. Natural spread of turnip yellow mosaic into a plot of healthy turnips; strip no. 1 is nearest to the source of infection. Each strip is 28 ft. by 6 ft. 6 in.

A plot put out in 1947 to study the rate of spread under natural conditions was a failure due to drought, but infections were found at the end of the season, not only at the extremities of the plot, but also in neighbouring experimental turnip plots belonging to other workers.

MATERIALS

Turnip yellow mosaic virus has been prepared from a number of cruciferous plants, namely radish, *Brassica carinata*, turnip, Chinese cabbage and from a specimen of broccoli found infected naturally. For general use either turnip or Chinese cabbage is suitable and, generally speaking, old or 'hard' plants give better yields than rapidly growing 'soft' plants. Yields of up to 1 g. of virus per litre of sap have been obtained from stunted turnip plants grown in the open, but the usual yields from glasshouse plants vary from 200 to 500 mg./l. On the other hand, Chinese cabbage plants grown in beds of soil under glass and supplied with plenty of water and minerals rarely give yields which justify the labour of extraction.

METHODS

(1) Dry weights of materials were obtained by drying solutions overnight at 110° C. in small-capped counterpoised weighing bottles. After cooling in a desiccator over phosphorus pentoxide, they were weighed on a microbalance. All determinations were made in triplicate.

The method of freeze-drying, which is very convenient for many virus preparations, cannot be used as the cohesion of the fluffy material obtained by this technique is so slight that it has not been found possible to release the vacuum in the desiccator without losing much of the material, even when the air is admitted through a wash bottle containing concentrated sulphuric acid at a rate of only one bubble per second.

(2) Nitrogen determinations were made by the micro-Kjeldahl method using about 0·5 mg. nitrogen and titrating the solutions of ammonia in boric acid with hydrochloric acid. The determinations were usually made in quadruplicate.

(3) In most cases phosphorus was determined gravimetrically as strychnine phosphomolybdate.

(4) Pentose estimations were made by means of the Bial reagent as modified by Militzer (1946). Xylose, purified yeast nucleic acid, and tobacco mosaic virus of known phosphorus content have all been used as standards. Xylose is a satisfactory standard but only if the heating at 100° C. is carried out for more than 8 min. instead of the three recommended by Militzer, as the pentose in nucleic acid does not react so rapidly with the reagent as does free pentose. The colours were measured photoelectrically, and the quantities of reagents and materials were adjusted to allow estimation of 50–100 μg. of purine bound pentose.

The method of Mejbaum (1939) was not found suitable for quantitative work, nor was the method of Tillmans & Phillipi (1929).

PURIFICATION METHODS

The original method described by Markham & Smith (1946) is quite reliable at certain times of the year, but fails to give preparations in the spring and summer months for reasons which will be mentioned later. Accordingly, a new method was devised which rarely fails to produce a crystalline preparation in a few hours even in the hands of unskilled persons.

Plants infected with the virus are minced, either fresh or after freezing overnight, and the sap is

expressed. If field turnips are used it is necessary to use a hydraulic press to remove the last half of the sap. The pH of the sap is usually about 5·7. The sap is clarified by adding slowly with stirring 300 ml. of 90 % ethyl alcohol to each litre. This quantity of alcohol is fairly critical and it should be measured carefully.

The copious precipitate which forms is centrifuged off at once at low speed (15 min. at 3500 r.p.m.). To the supernatant liquid a volume of saturated ammonium sulphate equal to half the volume of the clarified sap is added and the solution is allowed to stand, preferably overnight. After a few hours large numbers of small octahedral crystals are to be found in the liquid and these increase in size with time. Many small, highly refractile birefringent crystals are also to be seen, especially in the sap from old turnip plants, but these are inorganic and are removed later.

The crystalline precipitate is centrifuged off (30 min. at 3500 r.p.m.), but the supernatant liquid frequently deposits a second crop of crystals on standing for a day or two, and so may be kept. In addition it is very difficult to remove all the small crystals by centrifuging, as they sediment rather slowly because they are not very dense.

The precipitate which contains the virus and much insoluble material is resuspended in a small volume of water (one-tenth to one-quarter of the original sap volume) or buffer solution and is centrifuged again to remove insoluble material. Precipitation of the virus followed by resolution of the crystals and centrifuging to remove debris is repeated several times. In favourable circumstances the virus preparation may be fairly clean by this stage.

The other methods of purification which have been tried have been less successful. The sap may be clarified to some extent by the addition of 2–4 % of disodium phosphate or by heating to 55–60° C. and the virus can be precipitated as crystals from such solutions by the addition of salts. A large amount of contaminating material remains, however, and it is difficult if not impossible to remove. Clarification of the sap with alcohol as a preliminary to the purification would appear to be essential.

Further purification

Several methods may be used for continuing the purification. The virus is not digested by trypsin and treatment with commercial pancreatic extract (10 mg./ml.) and incubation for a few hours with a few drops of chloroform as preservative will remove some of the contaminants. The enzyme is removed by several recrystallizations of the virus.

A treatment which is of use in removing brown pigment if present is crystallization of the virus from dilute alcohol solutions. A solution containing some 5 mg./ml. of virus and a trace of salt is cooled to 0° C., and 0·25–0·30 volume of absolute alcohol is added gradually with stirring. On adding drop by drop a solution containing 20 ml. of absolute alcohol, 10 ml. of glacial acetic acid and water to make 100 ml., the virus solution becomes turbid, and this turbidity disappears on allowing the solution to warm. If the solution is left in a refrigerator for a few hours the virus crystallizes out in the form of small birefringent needles, which may be centrifuged off in the cold (when freshly formed they may dissolve at room temperature) or, better, they may be left at 1° C. for a few days, when the mass of crystals will be found to adhere to the bottom of the container and the mother liquor may be decanted without difficulty. The crystals are then dissolved in M/10 disodium phosphate or a neutral buffer, as they form cloudy suspensions in plain water. Spinning at 5000 r.p.m. removes the pigmented material which by now is insoluble.

The mother liquor from the alcohol crystallization diluted with water is found to have a pH of about 3·7 which is close to the isoelectric point of the virus.

GENERAL PROPERTIES OF THE VIRUS

Turnip yellow mosaic virus prepared as above forms colourless opalescent solutions. Electron micrographs of the particles deposited from such solutions (at about 0·1 mg./ml.) show that they are approximately spherical with diameters of about 20 mμ (Pl. V, fig. 2). This shape is to be expected from the type of crystals deposited from salt solutions.

When $\frac{1}{4}$ vol. of saturated ammonium sulphate solution is added, the virus is deposited as small isotropic octahedra, frequently with cube faces also developed (Pl. VI, fig. 1). Often the crystals are badly formed and tend to a spherical shape. Larger crystals may be deposited from dilute solutions (less than 5 mg./ml.) if saturated ammonium sulphate is added gradually until a turbidity just develops. This turbidity clears on cooling, suggesting that the virus may begin to come out of solution in an amorphous form as does tomato bushy stunt virus (Bawden & Pirie, 1938), but this reversal does not take place after a few minutes' standing, suggesting that crystallization has already started. No amorphous deposits have ever been obtained and in this respect the virus is quite exceptional. Octahedral crystals are invariably deposited from salt solutions and crystallization has been effected by sodium thiosulphate, magnesium sulphate, sodium chloride and other salts, as well as ammonium sulphate, which has given the largest crystals up to the present. The crystals from alcoholic solutions, on the other hand, tend to be birefringent needles deposited in hedgehog-like clumps (Pl. VII, fig. 3), but on occasion

nearly circular laminae have been observed. The latter were deposited from strong solutions of virus and when isolated and diluted crystallized in the more usual form.

The structure of micro-crystals deposited from solution (Pl. VI, fig. 2) has been studied by Cosslett & Markham (1948) by means of the electron microscope. Assuming that the particles were perfect spheres, in point contact, they obtained the value of 19·5 mμ for their diameter, but this is probably an underestimate as the particles may approach each other more closely and so have considerable areas in contact with each other.

The virus has been found to be soluble in dilute buffers having pH's from 3·5 upwards, but if solutions are dialysed exhaustively and then dialysed against weak acetic acid, the virus comes out of solution and then needs to be neutralized before it will form clear solutions again. A solution has been left in M/10 acetate buffer of pH 4·5 for over a year at room temperature without the virus protein losing its ability to crystallize.

The virus is apparently unaffected by being frozen at −10° C. for 20 min. in an aqueous solution.

CHEMICAL PROPERTIES OF THE PREPARATIONS

The virus prepared by the method described gives the usual colour reactions for proteins, and in addition gives the Molisch reaction for carbohydrates.

Quantitative analyses have been made as a routine on most preparations and they have been surprisingly uniform considering the complexity of the preparations, which will be discussed in a later section.

The nitrogen content has been found to be from 14·4 to 15·3 %, while the phosphorus content has only varied from 2·13 to 2·24 % from the time that it was decided to use the accurate gravimetric method for the determinations. The nucleic acid, which appears to comprise some 22 % of the total weight of the preparations, accounts for all the phosphorus and carbohydrate present, while 72 % of the total has been isolated as nucleic acid-free protein after alcohol denaturation.

The quantity of phosphorus liberated by mild acid hydrolysis in normal sulphuric acid for 1 hr. is almost exactly half of the total, being 1·1 % and approximately equivalent to the purine-bound pentose estimated by the Bial reaction, which is 5 % (theory 4·85 : 1, found 4·55 : 1).

Tests for desoxyribose have invariably been negative.

The ultra-violet absorption of the solutions is characteristic of nucleoproteins and has a maximum at about 263 mμ (Text-fig. 2*A*).

INFECTIVITY AND SEROLOGY

Purified preparations have been tested on Chinese cabbage plants and have been found highly infectious (Table 4). As the virus does not give visible local lesions consistently a detailed infectivity study has not been carried out. Infectivity is lost fairly rapidly at room temperature and after a few weeks solutions may be quite uninfectious.

Table 4. *Infectivity of purified virus preparations*

Virus (g./ml.)	Plants infected
10^{-7}	8/8
10^{-8}	3/5
10^{-9}	2/8
10^{-10}	0/8

Table 5. *Reaction of virus with antiserum prepared by intravenous injection of 2 mg. of purified virus*

Virus concentration (g.)	Antiserum dilution		
	1:50	1:100	1:250
10^{-4}	+++	++	−
10^{-5}	++	++	+
10^{-6}	−	−	−

Antisera to the virus have been prepared by injection of quantities of about 1 mg. of purified material intravenously in rabbits and these react at dilutions of 1:250 or more (Table 5). Tested with this serum diluted 1:50, $5{\cdot}9 \times 10^{-6}$ g. of the virus gave a visible precipitate in a total volume of 2 ml., while $5{\cdot}9/\sqrt{(2)} \times 10^{-6}$ g. gave no precipitate. The precipitates are compact and resemble those from other spherical viruses.

The serology of this virus is being studied in detail and will be published elsewhere (Matthews, 1948, and unpublished).

THE ACTION OF ALCOHOL ON THE VIRUS

The action of alcohol on this virus is so unusual that it has been studied in detail.

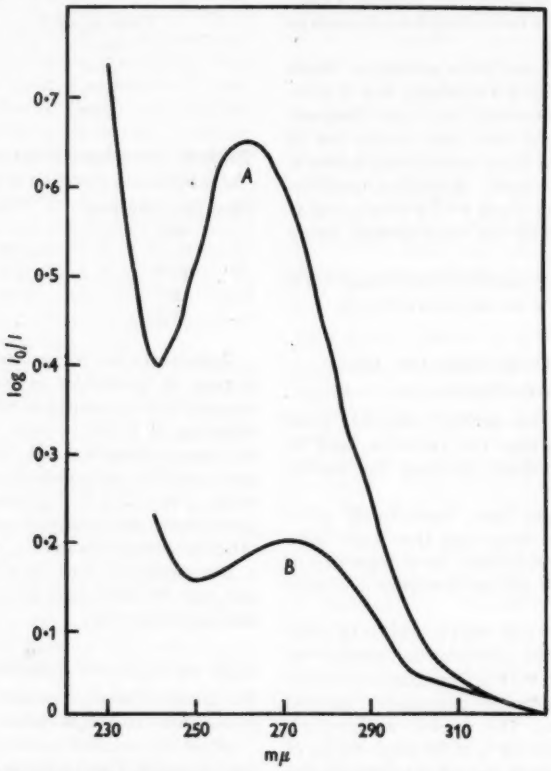
When the original method of purification (Markham & Smith, 1946) was adopted there was no doubt that the virus could be precipitated out of infective sap by 25 % alcohol at room temperature in winter. Difficulties arose with the arrival of warmer weather and a new method had to be devised.

Preliminary tests had shown that treatment with alcohol was the most effective method of clarifying the sap of the cruciferous plants used, and an investigation of the optimum quantity for this was carried out. To 200 ml. quantities of Chinese cabbage sap 50, 60, 67, 75 and 85 ml. of 87 % alcohol were added. The solutions were centrifuged after 4½ hr. and the supernatants had ammonium sulphate added to one-third saturation. The pellets were resuspended in water, spun and the supernatants also treated with

ammonium sulphate. Hardly any virus was present in these pellet extracts and they were discarded. The other solutions were worked up and good yields were obtained in the first three and nothing at all in the last solution. The first solution was not well clarified and so for subsequent use a concentration of 300 ml. of 90 % (nominal) alcohol per litre of sap was adopted as a routine, being sufficient to clarify the sap without loss of virus.

to the former, nucleic acid was precipitated as a characteristic flocculent mass. Evidently at room temperature 33 % alcohol denatures the virus and liberates the nucleic acid.

A further experiment was made. 53 mg. of salt-free virus in 2 ml. of distilled water had 2 ml. of absolute alcohol added at room temperature. No apparent change took place, but when the solution was warmed to 40° C. the solution turned to a clear



Text-fig. 2. Ultra-violet light absorption spectra of whole virus *A* and top component *B*, at a concentration of 0·1 mg./ml. in a 1 cm. cell.

In order to find out what was happening in sap when the higher alcohol concentrations were used, a specimen of purified virus, containing about 100 mg. in 10 ml. of dilute neutral buffer solution, had 5 ml. of neutralized absolute alcohol added gradually. On standing, it precipitated an insoluble white precipitate which was spun off. The supernatant liquid was found to contain all the pentose from the virus and practically no protein, while the insoluble residue contained protein but no pentose at all. On addition of a few drops of concentrated hydrochloric acid gel. On dilution with water no further change took place, but on the addition of 2 ml. of 0·9 % sodium chloride solution the solution became turbid and precipitated.

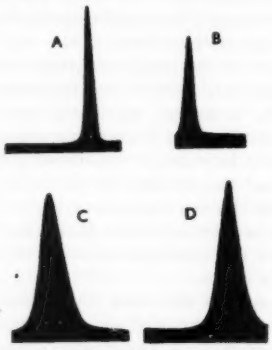
The denaturation of the virus by low concentrations of alcohol does not take place at low temperatures. Up to 67 % final concentration of alcohol has been added while the virus was kept in a freezing-bath at about −10° C., and the virus was recovered after careful dilution with water. This alcohol concentration is sufficient to precipitate the virus out of

salt-free solutions, while 57·5 % of alcohol will precipitate the virus at 0° C.

It was in the course of this investigation that the method of crystallizing the virus out of dilute alcohol solutions was discovered.

It is interesting that we have in this virus a source of nucleic acid which may be obtained in neutral cold solutions, and studies of this nucleic acid should prove profitable. Some preliminary experiments have already been made.

Nucleic acid prepared by splitting off the protein in dilute alcohol and concentrating by the addition of dilute mineral acid, dissolves in water in neutral or slightly alkaline solutions and forms water-clear solutions which are highly viscous and even gelatinous in concentrations of a few mg./ml. This suggests that the turnip mosaic virus nucleic acid is extremely asymmetrical and probably a linear polymer like that from tobacco mosaic virus (Cohen & Stanley, 1942) and thymus nucleic acid when prepared in a mild way.



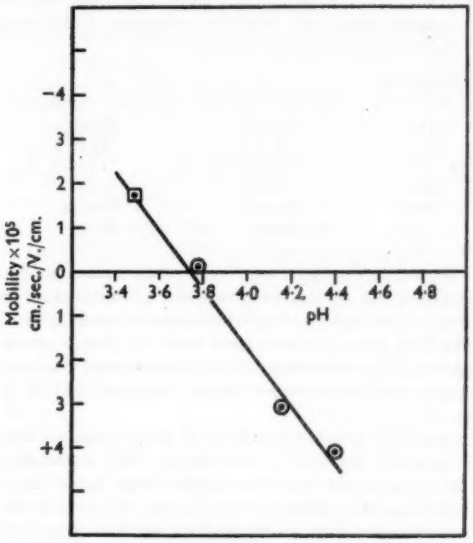
Text-fig. 3. Electrophoresis patterns obtained on the Tiselius apparatus: virus concentrations, 5 mg./ml. A and B, stationary at pH 3·78 after 3 hr. at 2·33 V./cm.; C, descending and D, ascending at pH 4·16 in a gradient of 5·48 V./cm. for 5 hr.

ELECTROPHORESIS EXPERIMENTS

Solutions containing 0·5 % of material have been examined in the Tiselius apparatus over the pH range of 3·50–5·38 in phthalate and acetate buffers of M/50 in the presence of M/20 sodium chloride. Over this range the virus migrates with a single sharp boundary, showing that the virus preparations are electrically homogeneous (Text-fig. 3). The isoelectric point is at pH 3·75 (Text-fig. 4).

THE NUCLEIC ACID-FREE PROTEIN OR 'TOP' COMPONENT

During an investigation of the physical properties of the virus preparations, details of which will be published later, Drs Ogston and Cecil found that in the ultracentrifuge the virus sedimented as two homogeneous components having different sedimentation velocities, the slower of the two consisting of some 20 % of the material and the faster of 80 %. Several preparations were examined with similar results, and it was at first thought that the virus existed as fourfold aggregates with a smaller quantity of single particles, this being quite consistent with the crystal form. A search of the electron micrographs failed to give any confirmation of this. Eventually, some 60 mg. of virus, partially fractionated on the Sharples supercentrifuge to increase the relative amount of the slower component, were ultracentrifuged in Oxford, using the separation cell of Tiselius, Pedersen & Svedberg (1937). From this material some 10 mg. of homogeneous 'top' component were returned.



Text-fig. 4. Electrophoretic mobility of turnip yellow mosaic virus.

Tested serologically, the solution reacted identically with the whole virus, both as far as the optimal ratios and the end-points were concerned (Tables 6 and 7).

1 mg. of the material in 2 ml. of water had 1 ml. of saturated ammonium sulphate added and it crystallized as octahedra indistinguishable from those of the whole virus (Pl. VII, figs. 1, 2).

A solution containing 0·01 % of top component was spectrographed in a 2 cm. cell and failed to give the characteristic nucleic acid absorption peak at 260 mμ. Semi-quantitative tests for pentose showed that the top component certainly had less than 0·9 % of pentose reacting with the Bial reagent, this

being the smallest amount which could be detected using the quantities of material available.

Table 6. *Top component and whole virus both initially at 0·1 mg./ml. compared at constant antiserum concentration (1:16), showing α-optimum and virus end-point*

Virus dilution	Top	Whole
1:1	30 min.	52 min.
1:2	12 min. α	13 min. α
1:4	17 min.	17 min.
1:8	33 min.	34 min.
1:16	120 min.	63 min.
1:32	> 180 min., < 24 hr.	126 min.
1:64	> 24 hr.	> 5 hr., < 24 hr.
1:128	> 24 hr.	> 24 hr.

Table 7. *Top component and whole virus compared at various antiserum dilutions, showing β-optimum and serum end-point*

Antiserum dilution	Top (0·1 mg./ml.)	Whole (0·1 mg./ml.)
1:1	8 min.	30 min.
1:2	3¼ min.	2½ min.
1:4	3 min. β	1¾ min. β
1:8	6 min.	4 min.
1:16	30 min.	52 min.
1:32	> 24 hr.	> 24 hr.

Photomicrographs of the crystals taken in the Department of Radiotherapeutics, Cambridge, in ultra-violet light with predominant wave-lengths of 265 and 254 mμ show that they are fairly transparent while crystals of the unfractionated material appear jet black under these conditions (Pl. VII, fig. 4).

As very small quantities of the material were available a detailed investigation of the interesting top component was impossible, but later when centrifuging a strong (1–2 %) virus solution to remove some pigment a boundary was found to form after some 20 min. spinning at 12,000 r.p.m. in a Sorval S.S. 1 centrifuge. Accordingly, the same solution was centrifuged in eight of the small celluloid tubes, each containing some 5 ml., in the hope that the material above the boundary would be enriched with the top component. After 2 hr. at 12,000 r.p.m. the tubes were carefully removed and a double boundary was visible in each tube (Pl. VI, fig. 3). The material contained between the boundaries was collected by means of a pipette with a fine tip bent at right angles. The solution so obtained was crystallized with ammonium sulphate, concentrated, and dialysed. Analysis showed that it contained only 28 % of the quantity of pentose in the same weight of the starting material.

Several other spins were made for times up to 5 hr. and it was found that the optimum time for the separation was 2 hr. In longer spins the boundaries became blurred, while in shorter spins the distance between the boundaries was too small for convenient sampling.

The total material from five 2 hr. spins was combined and spun in four tubes for 2 hr. Only one boundary was observed in this case, so approximately half the volume was pipetted off and this contained some 70 mg. of material. On analysis the Bial pentose was found to be about 0·8 %, this presumably being due to contamination with some 15 % of the bottom component.

Tested serologically the centrifuge fractions were indistinguishable from the starting materials. Spectra taken of the twice centrifuged material show a much lower peak absorption and the peak has shifted from 262 to 272 mμ, the trough at 242 mμ having moved to 249 mμ (Text-fig. 2).

For some time it was not known whether the top component was infectious as it is not always certain that local lesions will be formed on the test plants, while it would be difficult to show that the systemic infections obtained on inoculating numbers of plants with the best preparations of the top component were not due to the small quantities of the nucleoprotein inevitably present in these preparations.

Recently, however, we have been fortunate enough to obtain local lesions on a small percentage of the leaves inoculated with freshly fractionated material and the results of this first experiment suggest that the top component has no infectivity. There is, in fact, an indication that it may inhibit the infection by the nucleoprotein and this will be investigated further.

In the course of this work it was noticed that the pellets of virus obtained on centrifuging at high speed were crystalline. The crystals are not cubic and probably are much the same as those deposited from alcohol.

DISCUSSION

The turnip yellow mosaic virus which has been described above has proved to have some very unusual properties. The nucleic acid-containing fraction, which would be exceedingly difficult to obtain pure, would appear to have some 28 % of nucleic acid by weight in its composition, and even the mixed preparation has more nucleic acid in it than has been found in any other plant virus with the exception of that of tobacco ringspot (Stanley, 1939).

The extreme lability of the virus in 33 % alcohol has suggested that the nucleic acid-free material was produced by the preliminary alcohol clarification. It is usually impossible to prepare adequately purified material without the use of alcohol, but further treatment with alcohol has failed to reduce the amount of nucleic acid in the virus preparation without also denaturing the protein portion, and the

quantity of the top component in normal preparations appears to be reasonably constant, whether the original method of preparation or the new one is used, although the alcohol concentrations used in the two methods are different.

The serological and electrophoretic behaviour of the two components is very striking and suggests that the nucleic acid of this virus is held inside the particles, and that the external surfaces are identical in both cases. The nucleic acid-free material therefore probably consists of more or less hollow spheres with an empty space occupying some quarter of the total volume, as there is some reason to believe that the particles in solution are the same size (Cecil, Markham, Ogston & Smith, unpublished), while the dry particles are similar under the electron microscope and form crystalline aggregates with the same dimensions (Cosslett & Markham, unpublished).

Positive proof that the two components are antigenically identical is difficult to obtain as the sera used in the investigation have been made to mixtures of both components, and it is not possible to demonstrate the complete absence of one or other of the components in a fractionated solution, while it is known that a few microgrammes of the virus will cause demonstrable antibodies when injected into rabbits. The quantitative aspects of the reaction, however, show that the velocity of precipitation, equivalence of antibody to antigen and the virus and serum end-points are virtually the same, both for the unfractionated preparation, which is 80% bottom component and the ultracentrifuge preparation which is probably at least 90–95% top component. In addition, the serum which reacts with the latter can also be shown to precipitate the nucleic acid-containing material quantitatively (Matthews, unpublished).

While the top component appears to be a native protein, and in fact the only virus protein which has been obtained free from nucleic acid and still possessing all the qualities of a native protein, we believe that it is not produced by our method of isolation. One preparation made solely by repeated precipitation by means of ammonium sulphate, which we know does not affect the nucleoprotein, was centrifuged at high speed and a crystalline pellet was deposited together with flakes of pigmented material. By careful washing of the pellet with a small quantity of water, it was possible to dissolve some of the virus without much contamination with the pigment. On ultracentrifuging at Oxford this specimen was found to contain both components.

What is of great interest is the fact that this protein is not capable of multiplication, thus affording some experimental evidence for the belief that the presence of nucleic acid is essential for virus multiplication. A point to bear in mind about this, however, is the fact that the nucleic acid of the virus would not appear to be superficial. Evidence which may be obtained from the study of the microstructure of the two components may well tell us something about the way in which viruses multiply.

The ability of turnip yellow mosaic virus to crystallize in several different forms is not exceptional, as Cohen (1942) has found that tomato bushy stunt virus will crystallize in non-cubic crystals from certain solutions, but study of the crystals, particularly from the two components separately, will almost certainly prove profitable. Although it has been known for some time that various proteins crystallize from alcohol solutions this is the first virus to be found to crystallize under these conditions, and the method may prove useful in some cases where crystallization has proved difficult or impossible.

SUMMARY

A new virus affecting cruciferous plants is described and the name suggested for it is the *turnip yellow mosaic virus*. The virus is sap-transmissible and has a dilution end-point in extracted sap of 10^{-5}. The thermal inactivation point lies between 70 and 75° C.

The virus is not transmitted by insects with sucking mouthparts, but the insect vector in nature is a flea-beetle, *Phyllotreta* sp. More than one species of flea-beetle can transmit the virus.

Under experimental conditions the virus has been transmitted by the mustard beetle, *Phaedon cochleariae* and its larva; by the 'long-horn' grasshopper, *Leptophyes punctatissima* Bosc.; by the 'short-horn' grasshopper, *Stauroderus bicolor* Charp. and by the common earwig, *Forficula auricularia* Linn. Lepidopterous larvae have, so far, failed to transmit the virus.

The relationship of the virus with biting insects is discussed.

Turnip yellow mosaic virus has been obtained from several cruciferous plants as a nucleoprotein of the pentose type. It crystallizes from salt solutions as isotropic octahedra and from 20% alcohol at pH 3·7 as birefringent needles. On centrifuging out of solution the pellets are also crystalline. The virus preparation contains some 22% of nucleic acid.

The virus is denatured at room temperature in alcohol of concentration greater than 30% in neutral solutions, and the nucleic acid, which is a large polymerized particle, is liberated.

The preparations have been found to consist of 80% of a nucleoprotein and 20% of a nucleic acid-free protein which may be isolated by high-speed centrifuging. The latter has the same electrophoretic mobility and isoelectric point (pH 3·7) as the nucleoprotein; both crystallize in the same crystal form, and they are serologically identical.

The nucleic acid-free material does not seem to be infectious.

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EXPLANATION OF PLATES IV–VII

PLATE IV

Fig. 1. Longitudinal section through the oesophagus and foregut of the aphis, *Myzus persicae* Sulz., showing the sub-oesophageal valve which prevents regurgitation of food materials. × 200.

Fig. 2. Longitudinal section through the alimentary canal of an adult flea-beetle, *Phyllotreta* sp., showing the valve which, being between the crop and the mid-gut, does not prevent regurgitation of food materials from the crop. × 200.

Fig. 3. Longitudinal section through the head and foregut of a larva of the mustard beetle, *Phaedon cochleariae*; note the food material in the crop and its close proximity to the mouth. × 67.

Fig. 4. Seedling of Chinese cabbage infected with turnip yellow mosaic by means of the flea-beetle, *Phyllotreta cruciferae*; note the intense 'clearing' of the veins and the feeding marks of the beetle on the cotyledon in the foreground. × 1.

PLATE V

Fig. 1. Plant of Chinese cabbage showing the bright mottling characteristic of turnip yellow mosaic; note the local lesions on the inoculated leaf in left foreground.

Fig. 2. Gold shadowed electron micrograph of the turnip yellow mosaic virus.

PLATE VI

Fig. 1. Crystals of turnip yellow mosaic virus deposited on the walls of a flask from a salt solution; note the presence of cube faces on some of the octahedra. × 80.

Fig. 2. Electron micrograph of turnip yellow mosaic virus deposited from solution on to collodion film and transferred to a beryllium film. Small virus crystals have formed and their structure may be seen. × 37,000.

Fig. 3. Photograph of a celluloid centrifuge tube in which virus solution has been spun at 12,000 r.p.m. for 2 hr., showing boundary formation and separation of the 'top' component: photograph taken by the Tyndall light with tube illuminated from above. × 2·4.

PLATE VII

Fig. 1. Crystals of whole virus from ammonium sulphate. × 500.

Fig. 2. Crystals of 'top' component from ammonium sulphate. × 500.

Fig. 3. Crystals of whole virus from 20% alcohol. × 500.

Fig. 4. Crystals of whole virus (black) and 'top' component (transparent) mixed after crystallization from ammonium sulphate. Photographs by ultra-violet light; some of the larger crystals have been crushed. × *c.* 1000.

(*MS. received for publication* 13. I. 1948.—Ed.)

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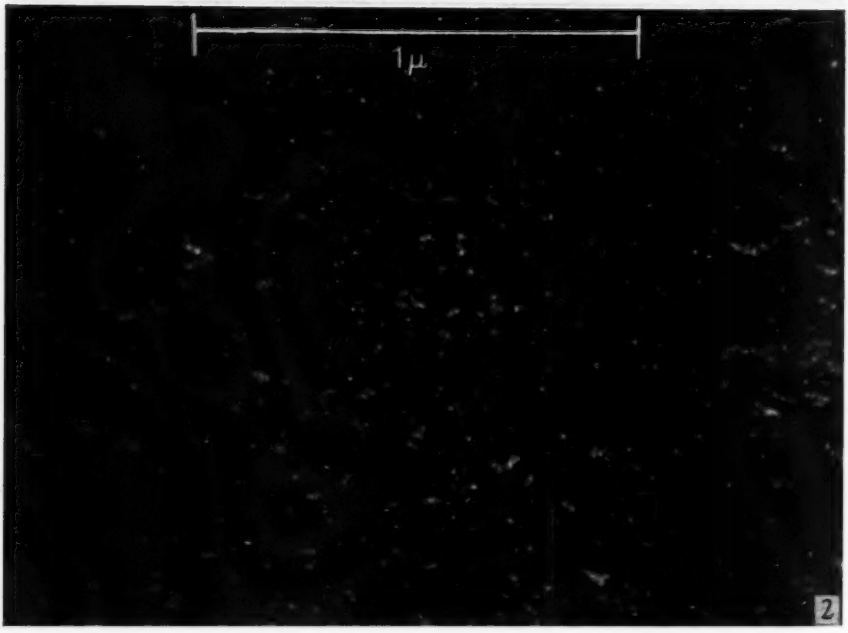

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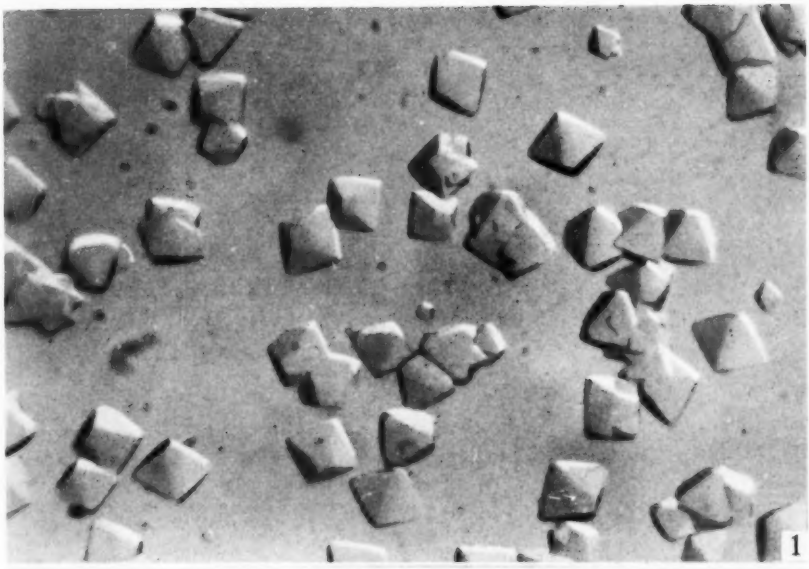

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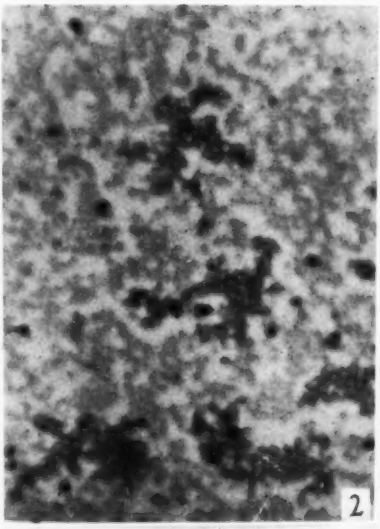

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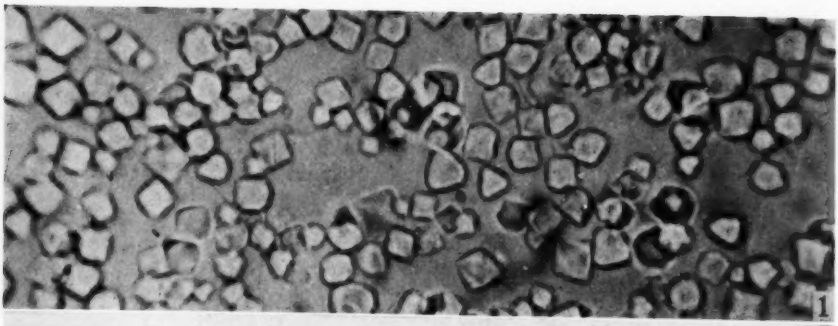
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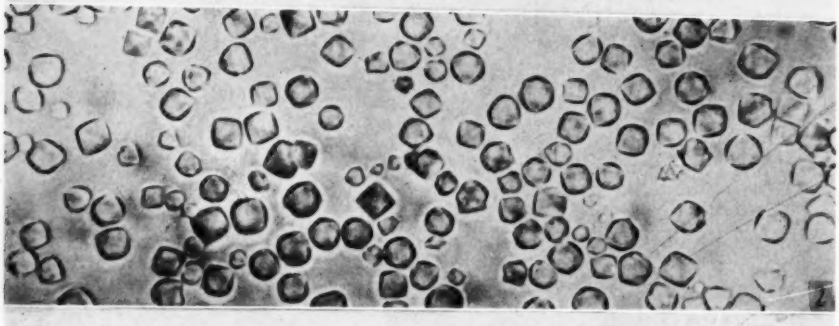
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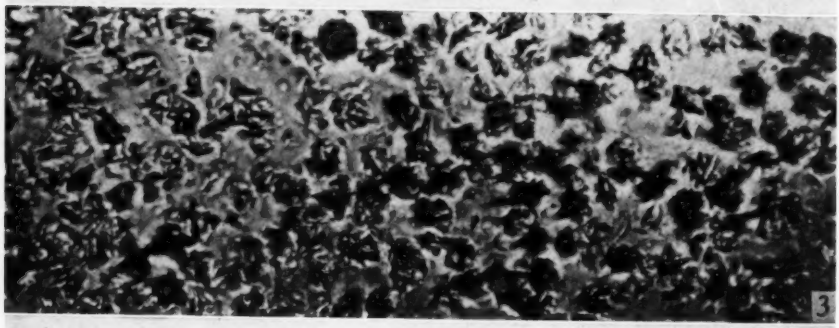
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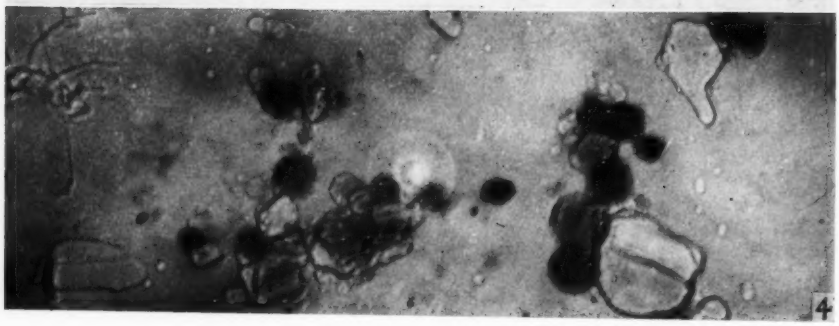
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